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A CYTOLOGICAL STUDY OF INFECTION OF BAART AND KANRED WHEATS BY PUCCINIA GRAMINIS TRITICI [1]

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INTRODUCTION

With the enormous increase in the work upon plant diseases, the need becomes apparent for greater knowledge of the nature of immunity and susceptibility to plant disease. Nowhere is this question of greater interest than in the study of the cereal rusts, where the problem of combating these diseases is recognized to be largely the task of breeding for rust resistance.

From the early days of Farrer's (*14*) speculations concerning the nature of rust resistance, down to the present, the problem has engaged the attention of many workers, but much still remains to be learned. The problem is being attacked by the geneticist, the chemist, and the cytologist. It is from the point of view of the last that the present study was undertaken, in the hope of learning something more of the actual behavior of the living host cell when attacked by the fungus and of the interaction of host and parasite.

Farrer (*14*) attempted to trace some connection between morphological characters and resistance, his idea being that a wheat plant with small stomata to keep the fungus from entering, or with very heavy epidermis through which the spores could not erupt to form pustules, or a wheat plant with narrow erect leaves on which the drifting spores could not lodge, would tend to be resistant.

Biffen (*3*), after careful breeding experiments with varieties of wheat, some of which were susceptible to, and others immune from yellow striperust, concludes that resistance and susceptibility are quite independent of anatomical features, for dissimilar plants may react similarly to the disease, and, what is even more conclusive, of two plants, similar in all anatomical details, one may be resistant, and the other susceptible. Moreover, immune plants were seen to bear small flecks and even abortive pustules, showing that the fungus entered, but for some reason was unable to develop normally.

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[3] Reference is made by number (italic) to "Literature cited," pp. 149–151.

With the abandonment of the theory that immunity is due to the exclusion of the parasite, recourse was taken to analogies with animal diseases, and it was assumed somewhat vaguely that resistance was due to "toxins and antitoxins."

DeBary (*2*) was one of the earliest workers to make careful microscopical studies of grain rusts. In 1866 he saw the germination of the urediniospores and the formation of the appressoria on the stomata. He noted the first flecks in six days and the earliest pustules on the eighth day. He also established the fact of heteroecism.

Eriksson (*7–12*), in field studies of rusts, found epidemics occurring where there was no apparent source of infection and became convinced that the disease was carried in some latent and invisible form in the seed and the plant growing from it. He found no recognizable mycelium in the growing plants, but thought he detected a thick plasma within the host cells which he believed to be a mixture of fungous and host protoplasm. According to him, when these infected plants approach maturity, the fungus ceases to be a symbiont in a latent condition and becomes an active parasite. The nucleus of an infected host cell becomes hypertrophied and represents a combination of fungous and host protoplasm. The nucleus then partially dissolves, setting free several "corpuscula nucleoli" of fungous plasm. The mycoplasm is now mature. The contents of these special corpuscles now pass out from the cell through minute pores in the wall and give rise to intercellular mycelium, leaving behind the empty corpuscle, which usually is elongated and surrounded by a broad "Lichthof." In the primary stage of the intercellular protomycelial life of the fungus there are no distinctly recognizable rust nuclei, but in the secondary mycelium arising from the first, the rust nuclei are distinct.

Eriksson's mycoplasm theory evoked criticism and in the lively controversy that followed several cytological studies were published describing in detail the vegetative mycelia of rusts on grains and grasses.

Ward (*28, 29, 30*), in his account of *Puccinia dispersa* Erikss. on bromes, figured and described with great detail and accuracy the germination of the spore, its entry through the stoma, the substomatal vesicle, the hyphae growing from it, and the development of haustoria. He maintained, and rightly, that what Eriksson supposed to be the differentiation of mycoplasm from the intimate mixture in the host cell, and its exit into the intercellular spaces to form mycelium, was really the entrance of the fungus into the host cell to form haustoria and that Eriksson in studying the haustoria in all their stages exactly reversed the sequence of events.

Eriksson grew grain from rusted seed in closed culture cases. A small percentage of the resulting plants were rusted. As no mycelium was to be found in the growing plants, this helped to convince him that the disease was propagated in latent and invisible form through the seed and the plant growing from it. Klebahn (*18*) repeated these experiments, using rusted seed supplied by Eriksson. No rust developed where the plants were kept isolated. More recently, Hungerford (*17*) grew plants from rusted seed under most carefully controlled conditions and obtained no rusted plants.

Miss Gibson (*16*) made interesting studies of the growth of rust spores on plants quite unrelated to their natural hosts. The spores germinated, were attracted to the stoma, and usually entered and made an initial growth. By the end of two days, or at latest four days, the fungus had

reached the limit of its development and was shriveled and dead. No haustoria were found. She concludes that—

> The facts seem to suggest that the death of the entering hyphae is not due so much to starvation as to some poisonous substance emitted by the cells.

Also that—

> The entrance of a stoma by any germ tube is no index of the capacity of that germ tube to infect the leaf.

Miss Marryat (*19*) studied the behavior of yellow striperust, *Puccinia glumarum* (Schm.) Erikss. and Henn., on a susceptible and an immune wheat. The fungus on the immune host enters in normal fashion, but the hyphae soon become watery in content, have few nuclei, and form almost no haustoria. The host tissue soon shrinks and begins to break down and die. Attempts to form pustules result in tangles of hyphae often lying deep in the tissues, among which a few abortive spores may be formed. The reason for immunity is not known but may be due to toxins and antitoxins.

Stakman (*26*, *27*) also made parallel studies of wheat stemrust on immune and susceptible varieties of wheat and also of oats inoculated with stemrust from wheat and barley, rye from orchard grass rust, and several others. The fungus on an uncongenial host enters as usual, and the first hyphae may be vigorous.

> Within a short time after the hyphae become closely appressed to the host-plant cells, there are usually unmistakable evidences of some deleterious influence upon the host cells (*27*, *p. 196*).

The plastids become irregular and are often clumped and soon become fainter, leaving a homogeneous, uniformly staining mass. Hyphae may grow past cells that escape injury. A part of a host cell may be destroyed, leaving the rest untouched. The harmful effect of the fungus may precede actual fungus invasion. The action is sometimes less sharp and rapid. The more resistant a form, the quicker are host cells and fungus killed.

> There seems to be a very definite antagonism between the immune plant and the parasite

and—

> immunity and resistance, especially when very marked, are quite independent of the nourishment of the plan t(*27*, *p. 198*).....The evidence would rather eem to favor the view that the whole problem is one of toxins in the host or parasite or, very probably, in both (*26*, *p. 46*).

METHODS

The strains of wheat stemrust used in the studies here recorded were *Puccinia graminis tritici* I and III and a local strain found growing in the breeding plot in Berkeley. The varieties of wheat were Baart or Early Baart (susceptible); Kanred, chosen for its immunity from several strains of stem-rust (*6*, *20*, and *23*); and to a minor extent Mindum (semiresistant). The seed of Early Baart used in this work was grown in the cereal plots at Davis in 1919, and that of Kanred came from Hays, Kans., in 1917 (C. I. 5146), and from plants grown here later from that seed, and the Mindum is Minnesota 470. The seedlings were grown in the greenhouse, inoculated about the ninth day after planting, kept under bell jars 48 hours, and then set in cheesecloth cages. The different lots of seedlings of Baart were grown and fixed in October,

December, and March; those of Mindum in October, January, and March; and those of Kanred in December, March, and July. The present studies are limited to infections on seedlings.

Material was fixed daily from the time of inoculation until 14 days after. The fixing fluids used were the chrom-acetic mixtures, Flemming's medium solution, and several modifications of Bouin's fluid. The latter proved unsatisfactory, in general, although a few fair preparations were obtained. Fixing fluids of ordinary strength served for the older tissues. Younger seedlings fixed better in solutions made up one-half or three-fourths strength. Flemming's triple stain was the one principally used.

INVESTIGATIONS

BAART

Baart, or Early Baart, is susceptible to the fungus and Mindum is somewhat resistant; but, as no differences were observed in the earliest stages of their development, two or three drawings of Mindum are included here. Drawings other than Early Baart are specified. Only the rust found in Berkeley and *Puccinia graminis tritici* I, were used.

The wheat leaf is parallel-veined, and over each vein on the upper side of the leaf is a small ridge running from one end of the leaf to the other, so that this surface of the leaf is minutely corrugated. The stomata are regularly arranged in a single longitudinal row on each side of every ridge about midway between its crest and the adjoining valley. The other side of the leaf is relatively smooth and its rows of stomata are about opposite those of the ribbed side. The stomata are all oriented alike, the long axis of the stoma being parallel to the length of the leaf.

The urediniospores were usually placed upon the ribbed side of the leaf by means of an inoculating needle and naturally lodged upon the ridges at a distance of several cells from the nearest stomata. Many of these spores germinated overnight, pushing out germ tubes through the pores in the cell wall. The germ tube hugs closely to the surface of the epidermis, conforming to the curve of each cell, and regularly takes the nearest route to a stoma. Perhaps the water vapor issuing from the stoma serves to attract the germ tube, or some faint odor diffuses out which acts as a stimulus for a chemotropic reaction. The living content of the germ tube flows along as it grows, leaving very little behind in the older part of the tube.

Once the stoma is reached, the tip of the germ tube swells and the protoplasm flows into it. It fits closely into the canoe-shaped hollow above the aperture of the stoma. When fully formed, the appressorium, as this pad or cushion of fungous protoplasm is called, is at least as large as the spore from which it came and contains nearly all of its living matter. The empty germ tube is now separated from the appressorium by a septum, and its delicate walls soon collapse. It is seen for a time as a hazy bluish line but soon disappears.

By means of this first step in the process of infection, the contents of the urediniospore are transferred with the greatest possible economy of material to the nearest point of entrance into the host plant. This process takes place rapidly, for leaves placed in the fixing fluid on the day following inoculation showed dozens of fully formed appressoria.

The young appressorium contains two nuclei, but older ones regularly contain four. Plate 1, A, shows a slightly oblique longitudinal section

of a stoma with its long femur-shaped guard cell containing plastids and the elongated dumb-bell-shaped nucleus, and a slant section through the adjoining accessory cell. At this stoma is a fully formed appressorium *a* with a withered remnant of germ tube still attached. Plate 3, D, shows a surface view of an appressorium on Kanred at the same stage. The two guard cells are flanked by accessory cells, and the appressorium is fitted closely into the hollow above the stomatal slit.

In spots where the spores were crowded on the leaf, slides were obtained showing several germ tubes competing for the same stoma. Plate 1, B, shows a longitudinal section of a stoma with three appressoria. The fixing fluid used for this preparation was a modification of Bouin's. The fluid proved to be too strong, but it is interesting to note that while the guard cell of the host shows pronounced plasmolysis, the fungi themselves are well fixed. This is perhaps an indication that the fungus has considerably higher osmotic pressure than its host. In this figure appressorium *a* evidently arrived first at the stoma, as it occupies nearly the whole hollow. Appressorium *b* came second and filled in one end of the hollow. It is evidently younger, being smaller and binucleate. Appressorium *c* is just entering, and its germ tube is forming a swollen club-shaped mass on top of *a*. The three germ tubes leading into these appressoria leave no question as to the separate origin of these three contestants for the place.

In nearly all cases the formation of the appressoria takes place with great regularity. A few deviations have been observed. Each of the little ridges running lengthwise of the surface of the leaf is reinforced at its apex by a slender band of heavy-walled lignified cells, studded at regular intervals by stout little thorn-shaped hair cells. In two or three cases an appressorium has been found wedged into the angle between the base of the hair cell and the adjoining epidermal cell. Plate 1, C, at *a* represents a cross section of one of these. The adjoining epidermal cell *b* is dead and collapsed. In another case (Pl. 1, D), taken from a longitudinal section of a leaf of Mindum, an appressorium formed on a dead collapsed ridge cell, failed to enter, pushed out a tube and grew to the other end of the host cell, and made a secondary appressorium there. Perhaps the dead host cell in each case allowed the diffusion outward of materials or of water from within the host, which served to attract the fungus. No appressorium has yet been observed on an ordinary healthy epidermal cell. These irregular cases form an argument against Fromme's theory (*15*) that "negative heliotropic reactions play an important part in bringing about the stomatal entrance of the germ-tube."

In entering the host plant the appressorium pushes a bladelike wedge through the stomatal slit, and the contents of the appressorium flow through, commonly forming a swelling inside which is termed the "substomatal vesicle."

Plate 1, E, shows a cross section of a stoma and the substomatal chamber. The stoma is occupied by the entering fungus. The collapsed germ tube and the appressorium can be seen outside the stoma and the beginning of the vesicle inside.

Plate 1, F, a longitudinal section of a Mindum stoma, represents a more advanced stage fixed on the second day after inoculation. It is drawn on a smaller scale than the preceding. The living content of the appressorium has passed through the stoma, leaving the delicate wall of the appressorium with its withered germ tube empty and wrinkled but not

wholly collapsed. Practically no substomatal vesicle was formed in this case, and the infecting hypha passed off directly at one end of the stoma. In the upper part of this drawing is shown the tip of an infecting hypha from another fungus which had entered an adjoining stoma.

Plate 1, G, also from Mindum, shows the fungus a day older. It is a longitudinal section cut obliquely through the stoma. The substomatal vesicle here is well developed and has given off two hyphae at each end, into which the protoplasm has moved, leaving the substomatal vesicle nearly empty.

Almost without exception the primary hypha or hyphae grow along the end of the guard cells and either run closely applied to the inner surface of the epidermis for a short distance in a direction parallel to the length of the leaf or, more rarely, slant down directly into the mesophyll. The nuclei and cytoplasm follow the growing tip or tips of the fungus, leaving the vesicle and the older portions of the hyphae near the stoma practically empty. This conserves the limited resources of the fungus until connection with the food supplies of the host is established by means of haustoria.

By the fourth day after inoculation the mycelium in Early Baart has attained a considerable size. The older hyphae near the stoma are empty or nearly so and the first two or three mesophyll cells attacked by the fungus are overgrown with hyphae and contain full-grown haustoria. From this center the mycelium spreads downward obliquely to the vascular bundle. As the hyphae work their way through the intercellular spaces they usually remain in contact with the walls of the mesophyll cells, following the irregular curved surfaces, but sometimes a hypha will cut across an open space.

Certain of the hyphae when unimpeded form long slender "runners." The long straight surfaces of the cells covering the vascular bundle form a particularly favorable place for this, and hyphae will run lengthwise of the leaf along these cells for considerable distances. Plate 2, A, at *a* and its continuation *a'* shows a relatively short runner. The tip of such a rapidly growing runner is slender and tapering, the cytoplasm fairly dense, and the two nuclei of the terminal cell are generally left behind and are to be found at some distance from the tip. These nuclei often are elongated, and the denser-staining portion inside the nucleus may also be considerably drawn out as if temporarily misshapen by the flowing onward of protoplasm to the narrow tip of the growing cell. Rust nuclei elsewhere are nearly isodiametric, being usually oval. Pole-Evans (*13*) saw these elongated nuclei of the runners and interpreted them as cases of direct nuclear division, but I have seen no evidence of this.

When the tip of a growing hypha strikes a host cell wall end-on or becomes wedged into an angle between two or more cells, or grows into a "blind alley" in the intercellular spaces of the leaf, it may make a haustorium. In fact, that is commonly the way in which the changes preparatory to the formation of a haustorium are initiated. It is not always essential that the growth in length of a hypha be forcibly checked, for a small minority of cases have been seen in which a haustorium was formed by a hypha whose tip appeared to be free to continue growth. Yet even in some of these cases (Pl. 2, D, and C, *b*) a careful comparison of the sections before and after the one in question may show that the obstructions were present in the plane above or below.

A hypha about to form a haustorium undergoes certain very characteristic changes, and as these cells are to be found by hundreds there is little doubt as to the details of the process. In Plate 2, B, the hypha, in growing up along the surface of one mesophyll cell, touched a second mesophyll cell obliquely at *b*, became deflected, and then the tip struck a third cell squarely at *c*. Growth in length is forcibly halted, the hypha has thickened, its contents are concentrated near the tip, and the two nuclei have moved up.

The next step in this process is seen in Plate 2, C. The hypha growing up touched a host cell first on one side, then on the other, and then the end struck a third cell at *a*. There was room enough to grow on, by bending, but growth in length was checked temporarily and other changes started. The slender-tipped growing hypha has now swollen into a broad club with its end closely applied to the wall of the host cell. The pair of nuclei which lag behind in a rapidly growing tip have moved up and have evidently undergone division. Two of the daughter nuclei have moved out into the swollen terminal portion. The other two are lying a short distance behind. The four daughter nuclei are nearly full grown and are about equal in size.

Soon after this a septum forms, thus giving rise to a short terminal cell. This is the haustorium mother cell. Plate 2, D, shows such a case and also A at *b* and *e* and C at *b*. This haustorium mother cell contains one of the two pairs of daughter nuclei. It varies considerably in shape according to the available space, but when relatively free it is oval.

There has been a marked change in the size of the nuclei. Before the septum formed, all four were approximately equal. Now the pair of nuclei in the penultimate cell are full grown, while those of the terminal cell have undergone a marked decrease in size. The nuclei are always present but are less than half of their former size. Not only is the nucleus as a whole reduced but also the densely staining body within it. Whether the dense spherical mass within the nucleus is a true nucleole or contains chromatin as well, I have been unable to determine. There is some evidence of a delicate chromatin network running through the nuclear cavity as figured by Blackman (*4*) in another rust, but these vegetative nuclei are minute and it is difficult to determine the details.

The penultimate cell now regularly pushes out one or more slender hyphae just below the septum. This is seen in Plate 2, D, where a small budlike branch is just pushing out, and in A, just below the haustorium mother cell at *b*, where the branch has already attained some length. The formation of a haustorium, then, does not stop the growth of a hypha permanently, for growth is practically always continued by means of these side branches.

This haustorium-producing cell has a broad surface contact with the host cell, and its contents have become very dense (Pl. 2, E). The branches below it in this case are slower than usual in forming but are beginning to push out. When the fungus is ready to enter, a minute pore is formed in the wall of the fungus cell and in the host cell wall next to it, probably by means of enzyms secreted by the fungus. The pore is ultramicroscopic in size; at least I have never seen it. Occasionally, however, at a later stage when the haustorium is formed, one finds a small circular red-stained spot on the host cell wall surrounding the point of entrance of the fungus. This suggests that the wall here

has been altered in composition, possibly because of the local spreading of the enzym that made the pore.

The formation of the minute opening in the two walls places the two osmotic membranes of host and parasite in direct contact, and the substance of the haustorium mother cell immediately begins to push through into the host cell (Pl. 2, E). As has been mentioned, the osmotic pressure in the fungus is probably higher than in its host, and this entrance may take place automatically by the extension of the fungous membrane as water passes into the fungus from the weaker solution in the host cell. This extension of the fungous cell into the host is accompanied by a corresponding invagination of the host protoplast. The appearance suggests strongly that the plasma membrane of the host is not broken but merely cupped by the invader.

A slightly larger haustorium is seen in Plate 2, F. The peglike haustorium of the earlier stage has now become differentiated into its two parts, the body, a dense globular mass showing no details of its contents, and the slender neck. The haustorium mother-cell is still dense in contents, and, as in Plate 2, E, there are concentrated darker staining masses within it.

Plate 2, G, is typical of a slightly later step in the process. The penultimate cell has its pair of full-grown nuclei and is pushing out branches just below the septum. Much of the contents of the terminal cell have passed into the haustorium, and in the less dense remainder we discover that the two small nuclei are still present. The young haustorium consists of a dense ball of fungous cytoplasm which stains a uniform deep red and a narrow neck joining this ball to the parent cell. The haustorium is still invested with host cytoplasm and still gives the impression of having stretched the peripheral layer of the cytoplasm of the host inwards and pushed it ahead into the central vacuole, forming a sort of pocket. If this impression is correct, the young haustorium is in one sense still outside of the living protoplast of the host, having merely indented its plasma membrane. The fact that the turgor of the host cell is not destroyed, but is, on the contrary, slightly increased, would also suggest an unbroken osmotic membrane.

Plate 2, A, *c* and *d*, shows a somewhat more advanced stage. The cell at *d* giving rise to the haustorium is now empty except for a rim of cytoplasm concentrated at its distal end near the point of exit into the haustorium. The haustorium *c* is larger and the two nuclei, or at least the nucleoles, are clearly visible in it. It is also beginning to expand, as is seen from the small vacuole within it. The rest of the haustorium is homogeneous in appearance.

Still later (Pl. 2, H) the parent cell appears to be empty, and the haustorium now contains the complete protoplast of a fungous cell. The haustorium has undergone a rapid expansion by the absorption of water from the host and now presents an appearance similar to that of the cell from which it came. The cytoplasm has much the same structure as before except that it is more open. One of the two nuclei is clearly visible in it.

Plate 2, I, shows an older U-shaped haustorium seen from the upper end of the U. The cytoplasmic investment is exceptionally heavy and includes a chloroplast at *a* and a small accumulation of cytoplasm about the neck. Looking into the ends of this haustorium, one sees that the peripheral portion is much more loose and open in structure than the center. Even the older, full-grown haustoria often have a denser core.

A noteworthy point in connection with this process is the fact that the empty haustorium mother cell remains plump. Hundreds of these cells are to be found in infected tissue, and while their shape may vary greatly according to the available space, they all give the impression of having turgor. This would suggest that when the cell contents entered the host cell to form the haustorium, at least a thin membrane was left behind, lining the cell wall of the mother cell, and that this membrane is continuous through the pore with the membrane of the haustorium inside the host cell.

It would be difficult to explain the passage of food from the haustorium to the mycelium through the empty cell if the latter does not possess an osmotic membrane, for its cell wall alone, if it is of ordinary composition, at least, would allow the escape of both the water and the food materials contained in it.

The host cell of Early Baart at this time shows no deleterious effects of the fungus.

In some cases, even in the half-grown haustorium, it is still possible to distinguish the two minute nuclei, or at least the nucleoles (Pl. 3, A). In the full-grown worm-shaped or branched haustoria (B and C), however, they can rarely be distinguished, although it is possible that they are still present.

The cell drawn in Plate 3, B (less magnified than the rest) was located near the center of a 6-day-old infection. Cells adjoining it were nearly filled with haustoria and surrounded by a felt of hyphae. The cell drawn contains two large haustoria, which are intimately associated with the living contents of the host cell, being covered with a rich layer of host cytoplasm. The infected host cell presents a flourishing appearance and may even have more plastids and cytoplasm than an uninfected cell.

A part of one of the large bundle-sheath cells from a 7-day infection is shown in Plate 3, C. The plane of the drawing was near the upper surface of the cell and includes some of the cytoplasm lining its upper wall. Three very large haustoria and one half-grown one are shown. At the left was an intricate tangle of hyphae not included in the drawing. It is not uncommon to find 8 or 10 full-grown haustoria in one of these large cells. The haustoria possess a delicate limiting membrane inclosing what appears to be fungous cytoplasm. There is often, but not always, a denser core. Nearly every haustorium, too, possesses one or more rounded clear spaces. These may be true vacuoles, or, in the living cell, they may have been filled with food material of an oily nature that was dissolved out during the preparation of the slide. The necks of these large haustoria in Plate 3, C, may have led to hyphae on the upper surface of the cell and may have been lost in sectioning.

Here, as in the younger material, the host cell gives every evidence of functioning normally, or even with somewhat heightened activity, and host and parasite seem fully congenial.

KANRED

The seedlings of Kanred given the same treatment as that described for Early Baart showed marked resistance to the local strain of rust. In repeated trials the fungus failed to produce flecks large enough to be visible to the naked eye.

Cytological study shows that the spores germinate readily on Kanred leaves. The germ tubes make their way directly to the stomata, where

typical appressoria are formed. Plate 3, D, shows a tangential view of a stoma with the fully formed appressorium fitted closely to its outer surface. A withered remnant of germ tube is still attached at *a*, the four nuclei are located near the center, and by looking down through the cytoplasm of the appressorium one can see the narrow stomatal slit. These appressoria are formed in great numbers, and when the spores are abundant on the leaf, two, three, and even four appressoria can be seen crowded together at a single stoma.

As was stated in a preliminary account of this work (*1*), relatively few of these appressoria pass through the stomatal slit in Kanred to form mycelium within the host. Six days after inoculation only 5 out of 100 appressoria had entered. Material taken 8, 10, and even 12 days after inoculation still showed numerous appressoria and relatively few infections. Moreover the plants were not uniform as to the percentage of entries. Of two plants grown side by side in the same pot and fixed at the same time, one might show very few entries and the other a percentage considerably above average. For greater accuracy, counts were made and the results presented in Table I.

TABLE I.—*Percentage of entries in Kanred at different dates after inoculation with rust*

Number of days after inoculation.	Total number of fungi counted.	Number of entries.	Percentage of entries.
6	100	5	5
8	133	14	10+
10	77	7	9
12	145	16	11+

Under the conditions of this experiment only about 10 per cent of the fungi enter. The other 90 per cent remain outside the stomata until they dry and fall off.

Plate 3, D, represents an appressorium fixed 6 days after inoculation. Eight days after inoculation the majority are still vigorous. A few, however, show signs of degeneration. Plate 3, E, represents a longitudinal section through a stoma on which at *a* is the shrunken appressorium with vacuolated cytoplasm and scarcely distinguishable nuclei. After 10 days the majority of the appressoria are withered, but a few (Pl. 3, F) are still vigorous. By the twelfth day under greenhouse conditions practically all the appressoria are withered and collapsed (Pl. 3, G). In the open, especially if subjected to a wind, the death of the appressoria probably would take place sooner.

A brief comparative study of Baart shows that the fungus enters much more readily. Four days after inoculation 26 out of 39 appressoria, or 67 per cent, had entered the host.

The stomata of living leaves of Kanred were studied and drawn during early afternoon of a bright day when some of the stomata under greenhouse conditions are wide open. These drawings were measured as to length and breadth of the stoma as a whole, and the slit was measured in length and width. Plate 4, A, represents the average of these measurements. Using the same methods, and the same magnification, an average stoma of Early Baart was drawn (Pl. 4, B).

A comparison of the two drawings shows several differences. The stoma of Baart is large and opens wide. That of Kanred is relatively slender and the aperture is smaller. In fact, the general epidermis of Kanred is finer in character, the ordinary epidermal cells being narrower than in Early Baart.

In Baart the fungus enters freely. In Kanred, under the particular conditions of these experiments, nine-tenths of the fungi are excluded. One would suppose that even the smaller stoma of Kanred, if fully opened, would permit the entrance of the fungus. It may be that the naturally small stomatal slit in Kanred is still further narrowed by stomatal action when an appressorium comes in contact with it. The presence of the appressorium might act as a stimulus by mere contact, by altering the gaseous exchange through the stoma or disturbing the moisture relations, by exerting a possible toxic influence upon the guard cells, or by its presence shutting off some of the light from the guard cells. It is at least conceivable that the guard cells might be sensitive to the appressorium and remain closed, thus excluding the fungus.

Concerning the effect of light, Pool and McKay (*22*) have shown in connection with their work on the relation of stomatal movement to infection by *Cercospora beticola* Sacc. that light is an important factor in stomatal activity, the stomata opening in strong light and closing in reduced light. Part of the material from which these counts were made was grown in December when the light is poor, and part in March when the light here is slightly above average. Both gave an average of about 10 per cent of entries in Kanred. Mindum and Baart, grown at the same times and in the same light and given parallel treatment throughout, showed the fungus entering much more freely. If the partial exclusion of the fungus is due to deficient light, or to some other factor in the environment, it is only in the smaller stomata of Kanred (of the forms so far studied) that this factor plays a decisive rôle.

In the foregoing studies a strain of wheat stemrust was used to which Kanred is extremely resistant. Other strains of the rust are known which can attack Kanred and produce pustules. One of these, *Puccinia graminis tritici* III Pers., was kindly supplied by Dr. Stakman, and a preliminary study has been made. The seedlings were grown, inoculated, and fixed in July, and by good fortune encountered the only hot weather of the season. Study of the slides shows that on the whole a larger percentage of the fungi enter. There is great variability among the plants, an occasional plant showing very few entries, and another as high as 30 per cent, the average being about 20 per cent. This is based on a study of only 8 plants and a total count of about 500 fungi. It remains to be determined whether the increase in the percentage is to be explained by the fact that host and fungus are congenial or by the fact that in this experiment the seedlings were exposed to greater light and heat.

In the experiments with the rust to which Kanred is resistant, 10 per cent of the appressoria effected an entrance. When a Kanred stoma is partly closed, the slit is widest near the ends. In the cases of entry in Kanred that have been observed, the fungus passes through the stomatal slit near one end and swells up inside, forming the usual substomatal vesicle, from which a normal infecting hypha grows along the inner surface of the epidermis to the nearest parenchyma cell. Here the hypha begins in normal fashion the formation of a haustorium in the mesophyll cell. The tip of the hypha forms a broad contact with the host cell, and

the usual septum appears some distance back, forming the cell that is to give rise to the haustorium.

With the entrance of the fungus into the host cell, however, making the first actual contact between the living protoplasm of host and parasite, abnormal changes begin. Plate 4, C, shows the first recoil from the contact. The haustorium-producing cell *a* is partially collapsed and has shrunken away from the host cell. There is a small red-stained spot on the host cell wall at the point of entry of the fungus. The scattered watery contents of the haustorium mother cell are in sharp contrast to the normal haustorium-producing cell. The latter at this stage (compare Pl. 2, A, at *d* and Pl. 2, G) would be turgid, closely appressed to the host cell, and the remainder of its protoplasm would form a dense layer close to the point of exit into the haustorium. The appearance here suggests that some substance diffuses out from the host cell, disorganizing the haustorium-producing cell. The harmful effect does not stop here, for the cytoplasm of the hypha below the mother cell has drawn back sharply from the septum, suggesting retreat from the advance of some plasmolyzing substance. It is not probable that this plasmolysis is due to fixation for, as pointed out earlier, the osmotic pressure in fungous tissues is high and they are rarely shrunken by ordinary fixing fluids.

Changes in the host cell are equally marked. The first change is an increase in turgor. By a fortunate mistake, one of the fixing fluids used proved too strong for the younger seedlings of Kanred. Preparations from it show the healthy mesophyll cells plasmolyzed, while the cells attacked by the fungus remain turgid, thus indicating an altered chemical condition in the host cell induced by the fungus. This excess of turgor in the attacked cell is rapidly succeeded by a collapse, as seen in Plate 4, C, and the protoplast may contract into separate masses, each of which may include plastids. Even the cell wall shrinks irregularly, leaving points on the outline of the cell. The nuclear contents are dissolved, although the nuclear membrane is distinct. Only in the immediate vicinity of the fungus are the plastids altered. There they appear as an indistinct mass. The adjoining host cells are perfectly normal in appearance.

A later stage in this reaction is seen in Plate 4, D, showing a longitudinal section through the epidermis and stoma. A portion of the collapsed appressorium is seen on the outer surface at *a* and the substomatal vesicle just inside the guard cell at *b*. Here, as before, only a single mesophyll cell at *e* was attacked. The disordered contents of this cell, the absence of the nucleus, the clumped, misshapen plastids, and the rough jagged points on the cell left by the irregularly collapsing wall give ample proof of the attack. It is to be noted in passing that in this case the collapse of the wall is sharply localized at *e*, and the end of the cell farthest from the fungus is relatively smooth in outline. The remaining stub of the infecting hypha, with its ragged discolored tip at *d*, lies at some distance from the host cell attacked. It may be that here, too, as in the earlier stage just described, the fungous cytoplasm retreated down the hypha to its base. Here a septum was laid in at *c*, walling it off. The relative abundance of cytoplasm and nuclei in the substomatal vesicle supports this view. The haustorium and the cell producing it and the portion of the hypha intervening between it and the stub have disappeared completely.

Plate 4, E, a tangential section of material taken 10 days after inoculation, shows the final stage of this process. The fungus evidently entered several days before the material was fixed. At *a* is seen a cross section of the projection from the appressorium that pushed through the stoma, and at *b* the hypha is seen. The part connecting *a* and *b* was cut off in sectioning. The hypha grew to the nearest mesophyll cell. At its distal end this hypha fades out and seems to be partially dissolved. Only one host cell was killed, and it is pale, having lost the intense affinity for stains possessed by newly killed cells. No structures are recognizable within the cell, and it is shrunken and no longer jagged in outline. A markedly thickened wall is to be seen at the contact between the dead cell and the healthy mesophyll cell adjoining it.

A fungus which has been checked in its first attempt to establish parasitic relations with the host may still possess enough vigor to grow. In Plate 4, D, the nucleus and cytoplasm certainly suffice for the production of a secondary infecting hypha and cases are to be found in which this has happened. Plate 5, A, is a section nearly tangential, showing the stoma and adjoining epidermis above and mesophyll tissues below. The fungus entered the stoma at *c* and formed two infecting hyphae. One at *e* attacked a mesophyll cell with the result that both the cell and the hypha leading to it died. Several slender threadlike projections on this dead host cell evidently represent the remnant of the irregular, jagged points seen on attacked mesophyll cells in earlier stages. A disintegrating remnant of the haustorium mother-cell is to be seen at the left of the dead cell near *e*. A shorter infecting hypha was formed at *b*. The killed host cell lies just above it and is found in the next section. These two attempts did not exhaust the fungus. A third hypha pushed out running along the edge of the stoma to *a*, and its tip some distance farther on contains a meager amount of living cytoplasm. The fourth and last attempt is at *d*, where a minute haustorium mother-cell is forming, equipped with thin cytoplasm and two nuclei so small and faint that they are scarcely distinguishable.

An extreme case of this sort is seen in Plate 5, B, where a fungus made no less than six distinct attacks. Two fungi entered this stoma, as may be seen from the two well-developed substomatal vesicles. Of these, one was quickly dispatched. A single infecting hypha was formed, resulting in a dead mesophyll cell at *b* and a dead discolored hypha at *a*. The other pushed off two primary infecting hyphae, numbers 6 and 1. With the death of No. 1, No. 2, a smaller, less vigorous hypha, branched out from its base. No. 2, in turn, died, and then came successively smaller and feebler hyphae, numbered 3, 4, and 5. Only the last two of these contain living cytoplasm. Nuclei, if present, are too small to be definitely distinguished. One can hardly believe that a single spore could provide sustenance for so long a struggle. It is possible that the same cytoplasm, retreating from one infecting hypha, takes part in the next attack. It is to be noted that there is a septum near the base of each one of these dead infecting hyphae which might mark the limit of the retreat as did the septum at *c* in Plate 4, D. It may also be that the reaction of the host against the fungus was a little slower in this case and that some slight nourishment was extracted by the fungus before the toxic reaction began.

Several of the host cells in this preparation have completely collapsed and others are dying. The walls of the latter are thick and stained red.

The central vacuole is obliterated, and the whole cell cavity presents a loose, dark granular appearance in which the plastids (some of them almost normal in size and shape) are faintly discernible. The nuclei have disappeared. Adjoining this pathological tissue are cells quite normal in appearance except for an occasional thickened wall.

These thickenings of the wall occur at the surface of contact between two cells as at *c* in Plate 4, E, and but rarely, if ever, on those parts of the cell wall which abut upon intercellular spaces. Usually the cell on one side of such a wall is pathological and the cell on the other side is healthy. There is some evidence that these thickened contact walls are impervious and prevent the diffusion of substances to and from the diseased cell. This may serve as a protection to the healthy cell by walling it off from any toxic materials formed in the pathological tissue.

In view of the fact that a vague impression exists among pathologists that the immune host attacked by rust somehow walls itself off from the fungus, thus checking its spread, it should be emphasized here that this formation of heavy walls between cells in no way excludes the fungus itself. So far as is known, rust hyphae do not enter cells nor pass from one cell into another. The only entrance into a cell is for the purpose of forming a haustorium, and this takes place from an intercellular space, and in Kanred at least, the host cell walls adjoining intercellular spaces are practically always left thin.

In some cases the reaction between host and parasite is more sluggish than in the examples described. The fungus makes a full-grown haustorium and gains enough food from it to enable the hypha to branch and grow on to the next cell before the first haustorium and the invaded host cell die. The new hypha makes a second haustorium and this may be repeated several times and further branching may occur. The interaction of host and fungus is slower in starting and not quite so severe in its effects. The result is a succession of dead, discolored hyphae and dead host cells and an ever weakening advance of the fungus. As many as two dozen cells or even more may be involved before the fungus dies.

In Plate 6, A, drawn on a larger scale than the preceding, is shown the advancing growth of the fungus. The dead cell at *e* already has succumbed to the attack of the fungus. At *d* is a nearly empty haustorium mother cell which has discharged its contents into the host cell to form the haustorium at *c*. The haustorium itself looks normal, but it lacks the cytoplasmic envelope of host cytoplasm. Evidently the normal relation of host and parasite has not been established. Still younger haustoria of the same mycelium show this cytoplasmic layer, but the older haustoria usually lack it. Perhaps some substance diffuses from the haustorium into the host cell which either destroys or repels the cytoplasm surrounding the haustorium. At *b* in the same figure (Pl. 6, A) is an older haustorium, the neck of which does not lie in this section. The advancing tip of the fungus at *a* has formed a typical and fairly vigorous haustorium-producing cell with dense red-stained contents and two minute nuclei, and, as usual, the hypha has branched below this cell. The host cell shows no further signs of disturbance. Its nucleus, which lies above the plane of the drawing in the same section, is unaltered in appearance.

Plate 6, B, shows a slightly older part of the same mycelium. At *e* is a full-grown haustorium which is somewhat vacuolated and ragged about the edge and has lost its connection with the cell at *d* that produced it,

but is still plump. At *a* is an empty haustorium mother cell still connected with *b*, the large worm-shaped haustorium produced by it. At *c* is a second empty cell and its haustorium. A considerable part of the contents of the host cell has collapsed into a mass about these two haustoria. The remainder of the cell looks normal. The host nucleus which is included in this shrunken mass shows decided signs of disintegration. Although this part of the host cell has collapsed from the combined effect of two large haustoria, the fungus itself does not seem to be harmed. The haustoria have not collapsed, nor is there any discoloration of the hyphae outside.

The results of a somewhat different balance of forces is seen in Plate 6, C, which represents a cell of the same mycelium but two or three cells away from those drawn in B. Here the host cell has not begun to collapse and shows no signs of deterioration due to the fungus, but the haustorium at *a*, which was evidently very large, has collapsed into a crumpled, irregular mass; the haustorium mother cell outside at *b* is wrinkled, flattened, and discolored, and the hypha below it is similarly affected for a short distance.

The details of the process may vary in different cases, but they come to the same end. Plate 6, D, is typical, with the dead host cell *e* stained a dense red and showing but little or no detail in cell contents, the collapsed haustorium mother cell *a* with its walls stained deep red, and the hypha devoid of cytoplasm below this cell and also for some distance up the branch springing from its base.

Although the reaction by which host and fungus are killed is considerably slower here than in the cases described earlier, it is still fairly rapid. Another mycelium, of the same age and found in the same leaf near this one, was completely killed. The dead host cells and an occasional dead hypha were all that remained.

In older material, all traces of the mycelium disappear except the initial hypha at the stoma. Just how this happens is not known. The hyphae can hardly be supposed to dry up, for the intercellular spaces of the leaf must form an excellent damp chamber. Moreover, the hypha nearest the stoma where some drying might take place is the one that persists the longest. In material taken 10 days after inoculation, no traces of intercellular mycelium were discovered.

DISCUSSION

Pole Evans (*13*), in a comparative study of the histology of the uredo mycelia of cereal rusts, notes characteristic differences between the species and tabulates his results as to the appressorium; the form, size, and septation of the substomatal vesicle; the number of the infecting hyphae; the size and form of the haustoria, etc. He says:

> These different species of *Puccinia* in the early stages of development of their uredo mycelia exhibit morphological characters (seen especially in connection with the formation of the substomatal vesicle) which serve at once to distinguish them from one another.

There doubtless are typical morphological differences between the vegetative mycelia of different rusts, but Plate 1, F and G, shows some of the difficulties involved in any attempt to classify rusts on this basis. We have here the same rust on the same host. Yet in one case there is practically no vesicle and only one infecting hypha, and in another there are a large vesicle and four hyphae. All gradations between the two can be

found. The fungus is decidedly plastic, and its individual vicissitudes are written in its morphology.

These observations on the behavior of an immune host attacked by a parasite are not extensive enough to warrant definite conclusions, but they are at least suggestive. The resistance of Kanred to this strain of stemrust appears to depend on two and perhaps even three distinct characteristics—one, the nature of its stomata by which under the conditions of this experiment, at least, all but a few of the fungi are excluded, another, the formation in the invaded host cells of a substance or substances fatal to the fungus, and third, the heavy cell walls between affected and healthy host cells, by which the spread of possible toxic materials into healthy tissues may be prevented.

Concerning the first point, one would suppose that a very narrow passage would suffice for the entrance of the fungus. Blackman (*4, p. 339*), in describing the process of fertilization at the base of the aecium in Phragmidium, tells how the nucleus passes from one cell to the other through a very fine pore. He adds:

> Neither before nor after its passage could a pit in the wall be observed.

Smith (*24*), in describing the formation of haustoria in Erysiphe, is impressed by the narrowness of the neck through which the nucleus and cytoplasm of the fungus pass to form the body of the haustorium. Eriksson (*11*) is concerned by the fact that the exit of the so-called mycoplasm of the cell into the intercellular spaces to form mycelium (or what is now believed to be the passage inward from the mycelium to the host cell to form the haustorium) must take place through an invisible opening in the wall.

> It effuses through the subtile pores that must be supposed to exist in the cell wall, that is to say, in the same way as the plasmodesmes between the cells.

But, perhaps, instead of comparing the entrance of the appressorium to the formation of a haustorium, or to fertilization, in which the fungus forms a very fine pore in a wall and passes through it, one ought rather to compare the appressorium in its tendencies and tropisms to the hypha, feeling its way sensitively along the intercellular spaces and easily checked in its course or deflected from it by obstructions. On this basis one would not expect the appressorium to pass through a much narrower channel than a hypha would enter.

In Kanred seedlings exposed to *Puccinia graminis tritici* I Pers., under the particular conditions of this experiment, but 1 fungus in 10 entered the stoma. This fact is of small importance so far as this strain of rust is concerned, for the immunity of this host is so great that even if all the fungi entered the host would not be appreciably harmed. However, this form of hindrance to the entrance of the fungus may not be limited to Kanred. Ward (*29, p. 37*) in his work with *Puccinia dispersa* Erikss. on bromes says:

> The vesicles and appressoria, infecting tubes, &c., may be visible much later than this however (i. e., four days), and it seems probable that infection can occur after delay, at least up to the sixth or eighth day, a point worth investigating.

The limited work with *Puccinia graminis tritici* III Pers. would seem to show that here, too, a large majority of the fungi are excluded. Even if only one-fifth of the fungi entered, however, it would not always mean that only one-fifth as much damage would be done by the parasite. It has often been noted in studying inoculated seedlings of wheat and oats that

where large numbers of competing mycelia are crowded in a leaf, the pustules formed are minute and produce few spores. The available food supply may be the main limiting factor. When, on the other hand, the mycelia are few and scattered, each attains to its full development and produces many spores. Moreover, in inoculations made by placing a single spore on a leaf of Early Baart, it has been noted that after the pustule has developed the mycelium spreads radially. Its extent can be determined by a slight discoloration of the leaf. Several days after the formation of the primary pustule, a circle (or ellipse, rather, for the growth is more rapid in the longitudinal direction) of secondary pustules forms around the margin of this spot. Given a free chance to develop, the damage done and the spore output resulting from the entrance of a single fungus into the host may be considerable.

If, then, many spores are used in inoculation and only one in five produces infection the damage to the host and the resulting spore output of the fungus is certainly more than one-fifth of what it would be if all had entered. When relatively few scattered spores are placed on the plant and only one in five produces a mycelium, the results would be approximately one-fifth what they would be if all had entered. The latter would be usual under field conditions; and if later it should be proved that the stomata of Kanred behave in the same way in the field as they do in the greenhouse, this characteristic would have some economic importance.

By becoming thoroughly familiar with the habits of the fungus in a congenial host, some light has been thrown on its history in an immune host. By learning to recognize the very regular and characteristic transformation through which a hypha goes before a haustorium is formed, and the appearance of the haustorium mother cell during and after the production of the haustorium, one learns to recognize the attempts of the fungus to form haustoria in an immune host and to see, in defeated attempts, the history written there.

The fungus follows a normal course of development up to the time when the parasite enters the host cell. When, in Kanred, there is evidence of at least the beginning of the formation of a haustorium, and there has been actual contact between the cytoplasm of host and fungus, both the host cell and the fungus near it die. But a hypha can skim the surface of a cell, or even wrap itself around it, and, so long as no haustorium is initiated, host and fungus are unharmed. Stakman (*27, p. 196*), as already mentioned, also noted that a hypha in an immune host could pass by cells without harming them.

When the reaction is slower after the entrance of the fungus into a host cell and haustoria succeed in growing to full size, more details of the process can be learned. The fact that the cytoplasmic envelope of the growing haustorium in Kanred disappears rapidly points to the possibility that the fungus may be giving out some substance into the host cell. Certainly the host cell undergoes chemical changes, whether due to the mere physical presence of the haustorium or to some substance diffusing from it, and these changes result in the death of the host cell. Moreover, some substance, or maybe more than one, diffuses from the host cell into the haustorium; and this results either directly or indirectly in the death of the haustorium, the collapse of the cell producing it, and the plasmolysis or death of a portion of the penultimate cell below. This

may happen while the haustorium is still small and even before the entire contents of the haustorium mother cell have entered the haustorium; or the action may be much slower, allowing full development of the haustorium.

There is irregularity in the balance of forces of the interaction between host and parasite, for in one case the host cell is severely damaged at a time when the fungus is not visibly harmed, and in another the host cell is still normal in appearance while the fungus has succumbed. Miss Marryat (*19, p. 136*) cites a nearly parallel case. In describing the general effect of *Puccinia glumarum* (Schm.) Erikss. and Henn. on American Club, a variety of wheat resistant to it, she says:

> Even hyphae which have managed to put out haustoria may become filled with small granules and their outlines appear so faint and indistinct that they seem on the road to complete disintegration. The host cells in such cases often appear moderately healthy, but in other portions of the leaf one may find hyphae which are still flourishing whilst the host cells in their vicinity are gradually dying, a response to a too vigorous onslaught on the part of the parasite.

Stakman (*26, 27*) also notes variability in the struggle.

Butler (*5*), in an excellent review of the literature on immunity in plants, sums up the evidence for a connection between acidity of cell sap and immunity, showing that in some diseases greater acidity favors fungous attack, in others it retards it, and in still others is indifferent. In yet other cases a change in acidity during ontogeny is accompanied by parallel changes in immunity. A form of wheat resistant to rust had more acid in its sap than the other varieties tested.

Wheat seedlings do undergo rapid chemical changes of some sort. One of the fixing fluids which plasmolyzed the younger seedlings fixed them well a week later. There is no evidence in my work at present that would connect this general change with rust resistance.

There seems little room in this particular case for the "starvation" theory of immunity tentatively discussed as one of the possibilities, by Ward (*28–30*), Marryat (*19*), Gibson (*16*), Spinks (*25*), and others. At every point of entry into a host cell the fungus is either killed back or driven back for a short distance. When the reaction of the host is somewhat deferred, the fungus makes a haustorium, and it evidently extracts food from the host—enough at least to let it grow on to new cells—and there is no evidence that this food is of an unsuitable nature. Here in these slower reactions we have, too, at least a hint that the harm done by the host to the fungus is due to a chemical reaction initiated after the entrance of the fungus into the host cell. The increased turgor of the host cell following its entrance points in the same direction. To be sure the fungus sooner or later exhausts itself in these unsuccessful attempts to enter into food relations with the host, but the observations recorded here suggest that in this case at least the failure of the fungus is due not so much to lack of proper food in the host as to a specific reaction set up there which destroys the fungus.

Ward (*28–30*), Marryat (*19*), Gibson (*16*), Orton (*21*), Stakman (*26, 27*), and others favor the theory that immunity in plant diseases is due to antagonistic reactions between host and parasite analogous to the formation of specific toxins and antitoxins in animal diseases, and the limited evidence presented here is in line with this.

SUMMARY

The germination of the spores and the formation of the appressoria on the stomata take place in the same way in the susceptible and immune hosts.

In Baart the fungus enters freely and grows rapidly. In Kanred, under greenhouse conditions, only a few of the fungi pass through the stomata; the rest remain outside until they shrivel and die.

In a congenial host, numerous haustoria are formed. A slender-growing hypha strikes a host cell, swells at the tip, its pair of nuclei divide, and a septum is formed, marking off a short terminal cell. This haustorium mother cell is closely appressed to the host cell, forms a fine pore through its wall and the host wall, and its contents, including both the nuclei, which have decreased in size, and the cytoplasm, now pass in, forming the haustorium. The osmotic membrane of the host appears to be invaginated by the haustorium, but apparently is still intact.

In Kanred the process is similar until a small haustorium is formed, which, either by its presence, or, as is more likely, by secreting some substance in the host cell, sets up chemical reactions within that cell, causing its collapse and death. The further diffusion of toxic substances into healthy host tissues is checked by the formation of thickened contact walls. One or more of the substances formed in the host cell diffuse into the haustorium, killing it, and causing collapse of the mother cell and the death and plasmolysis of the hypha back of it for some distance. If this reaction is rapid, the haustorium is destroyed while still very small; if more sluggish, a full-grown haustorium may be formed and some nourishment for further growth be extracted by the fungus.

Kanred possesses three means of defense against this strain of stemrust: stomata which shut out the majority of the fungi, the heavy contact walls adjoining pathological cells, and a true immunity. The observations recorded here are in line with the theory that immunity is due to definite antagonistic chemical interactions between host and parasite.

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PLATE 1

A.—Longitudinal section of stoma of Baart bearing a fully formed appressorium.

B.—Three appressoria at one stoma of Baart. The guard cell is plasmolyzed.

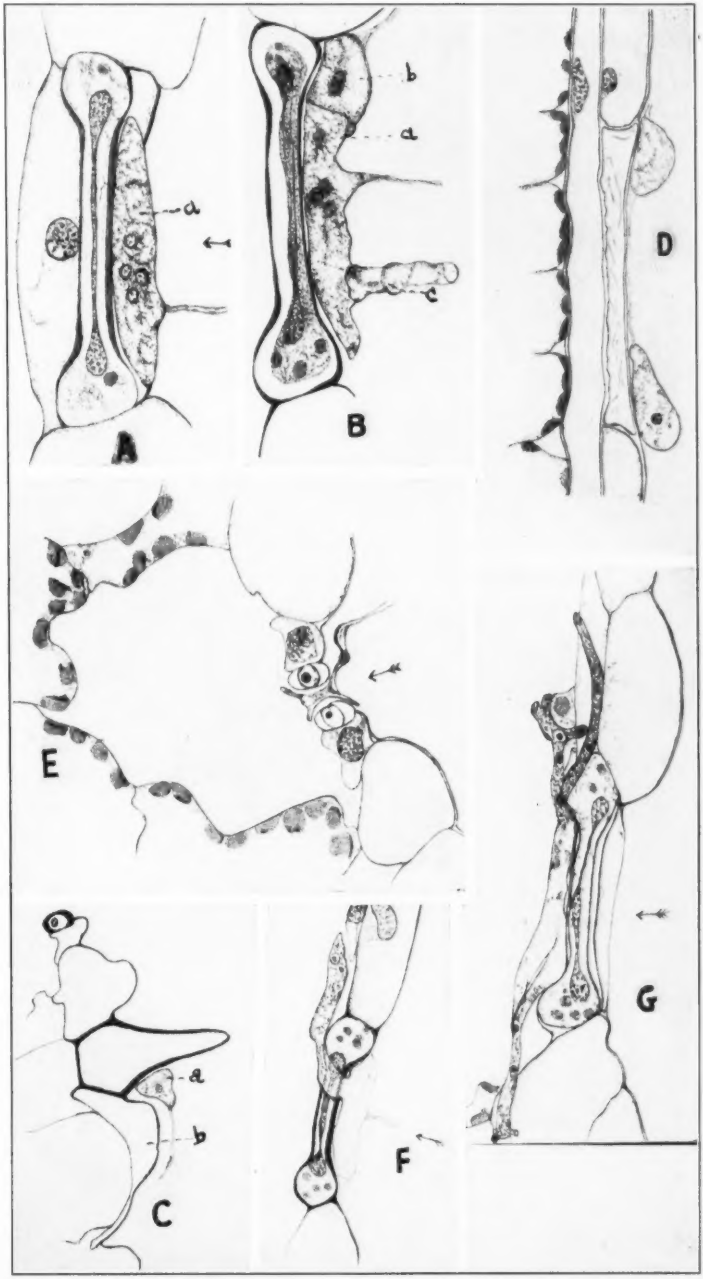
C.—Cross section of appressorium at a hair cell on the ridge of the leaf.

D.—Appressorium formed on a dead cell at the apex of a rib on a leaf of Mindum, a durum wheat. A secondary appressorium has formed from the first.

E.—Cross section of a leaf of Baart, showing stoma and substomatal chamber and the fungus in the act of entering the stoma.

F.—Longitudinal section of stoma of Mindum, showing empty appressorium connected with the infecting hypha within. No substomatal vesicle formed.

G.—Oblique longitudinal section from Mindum, showing large substomatal vesicle and four infecting hyphae.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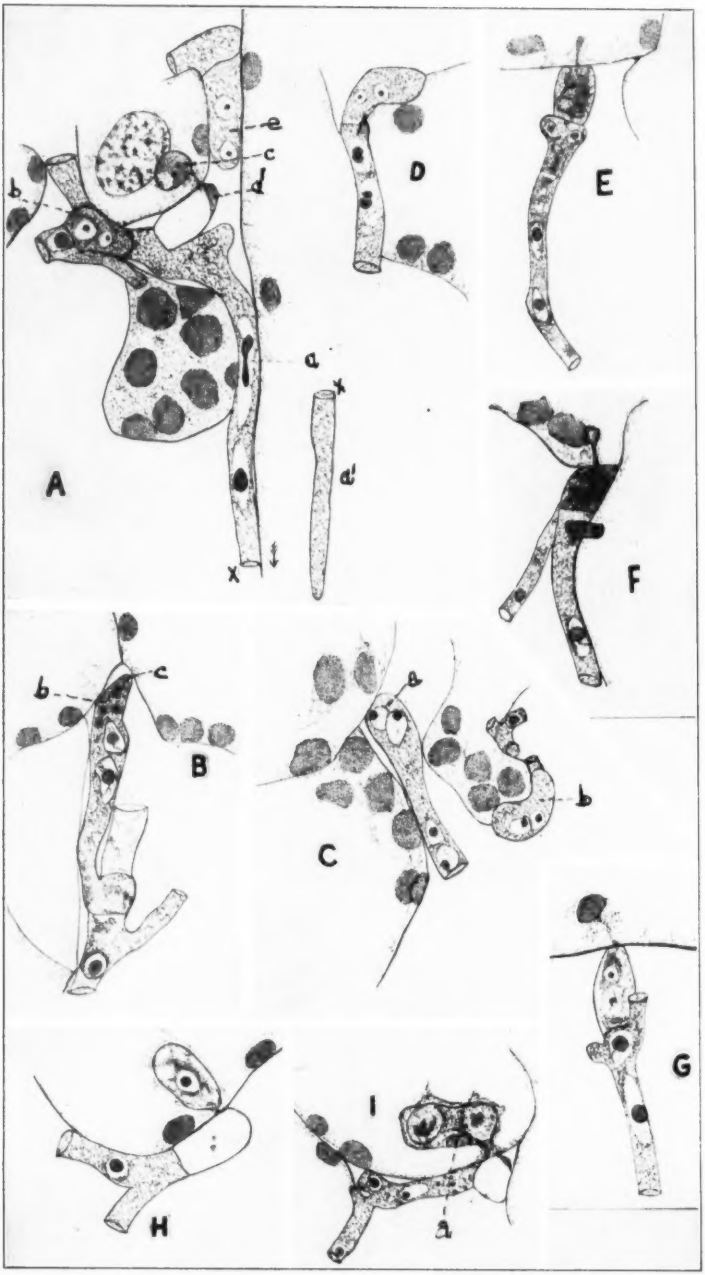
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PLATE 2

A.—Portion of 4-day mycelium showing a "runner" at *a* and *a'*, haustorium mother cells at *b* and *e*, and a young haustorium at *c* forming from the cell *d*.

B.—Hypha, forcibly checked in growth by striking host cell, preparing to form haustorium.

C.—Similar but slightly older. The tip of the hypha is swollen, the pair of nuclei have divided, and one daughter pair has moved to the tip. At *b*, a slightly later stage.

D.—Septum formed a short distance back from the tip of hypha, forming the haustorium mother cell. Its nuclei have decreased in size. Branch forming from hypha behind.

E.—Beginning of haustorium formation. The young haustorium is a peglike projection into the host cell. Mother cell very dense.

F.—Slightly older haustorium differentiated into neck and body. Mother cell still dense in content.

G.—Larger haustorium, still invested with a sheath of host cytoplasm. Contents of haustorium mother cell less dense. Its nuclei much smaller than those of hypha behind.

H.—Entire contents of haustorium mother cell emptied into haustorium.

I.—Larger haustorium with denser core and heavy envelope of host cytoplasm, including a plastid at *a*.

Drawings were made from preparations of *Puccinia graminis tritici* on Baart.

PLATE 3

A.—Baart. A half-grown haustorium and the empty cell from which it arose. The nucleoles of the nuclei still visible.

B.—A cell of Baart (drawn on a smaller scale) showing two full-grown haustoria. Material fixed 6 days after inoculation. Host cell not visibly damaged.

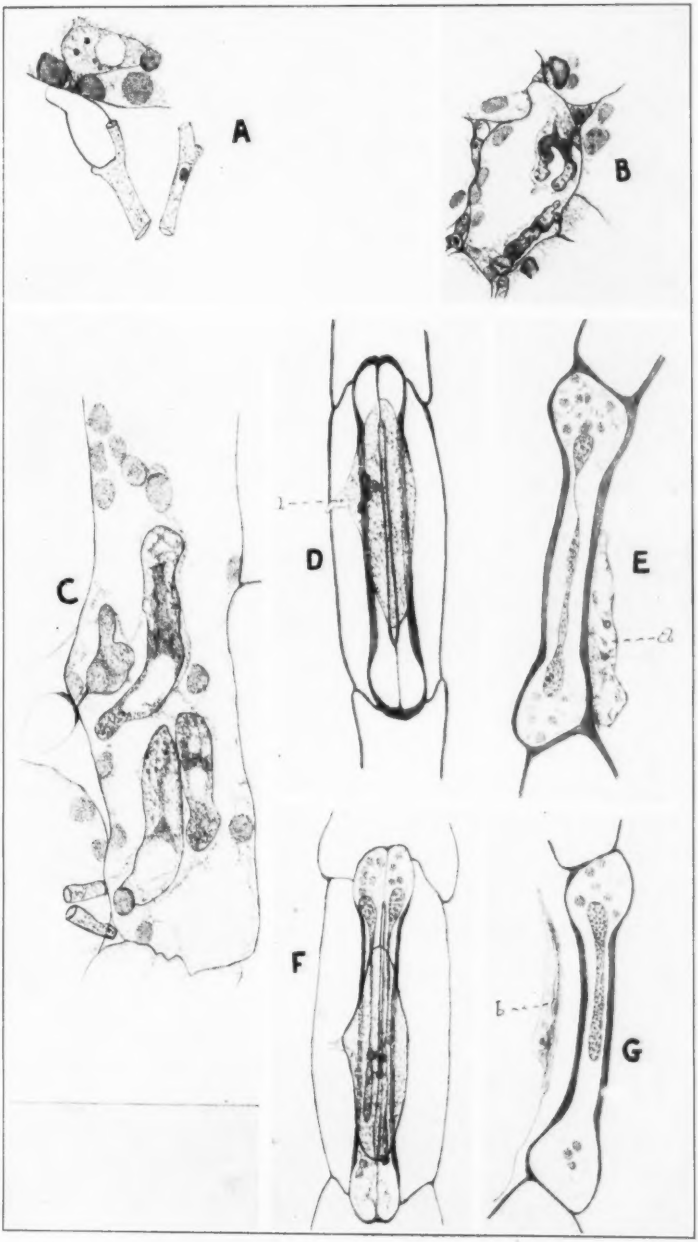
C.—Baart taken 7 days after inoculation. Cell of bundle sheath containing one half-grown haustorium and three full-grown ones. Host cell still normal.

D.—Appressorium on stoma of Kanred, 6 days after inoculation. Tangential section.

E.—Longitudinal section of Kanred stoma 8 days after inoculation, bearing an appressorium which is slightly shrunken.

F.—Tangential section of stoma and appressorium 10 days after inoculation.

G.—Longitudinal section of Kanred stoma bearing withered appressorium 12 days after inoculation.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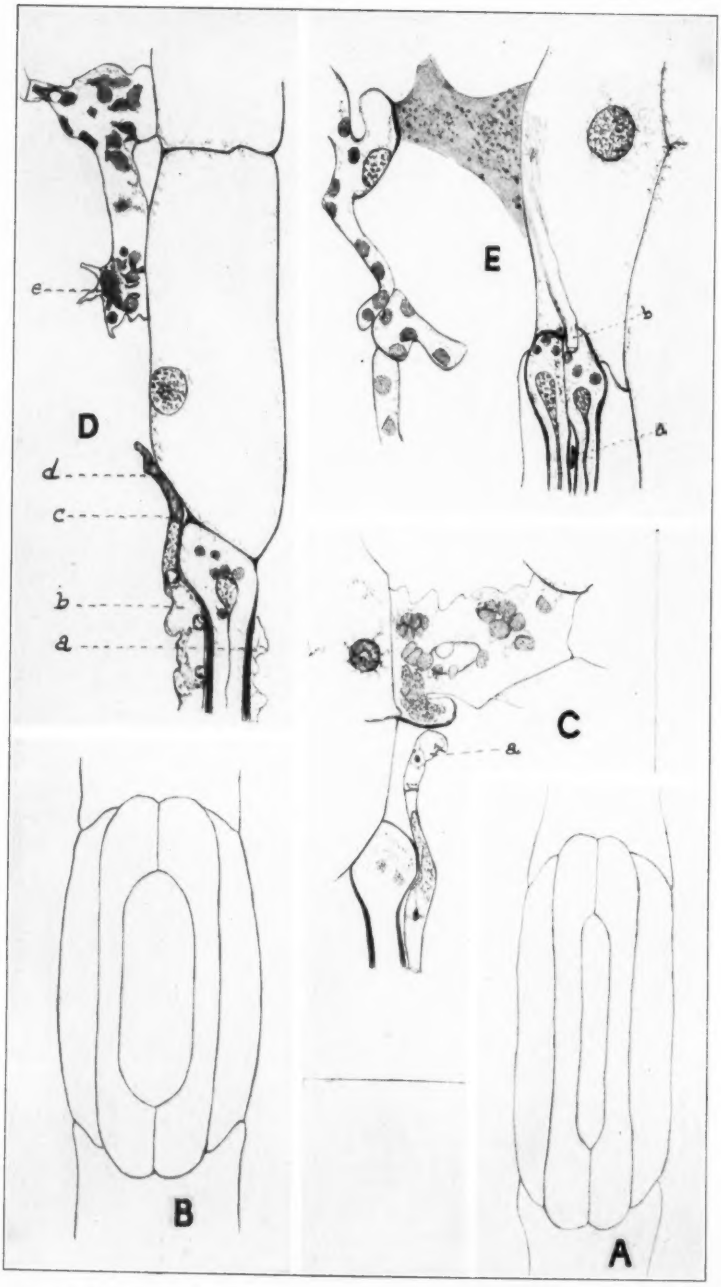
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PLATE 4

A.—Diagrammatic drawing of Kanred stoma representing the average of the measurements of 20 camera drawings of stoma.

B.—Similar drawing representing the average Baart stoma.

C.—Infecting hypha after attacking a mesophyll cell of Kanred. The contents of the host cell are collapsed, and its nucleus nearly dissolved. The haustorium mother cell *a* is shrunken, and the hypha below is plasmolyzed.

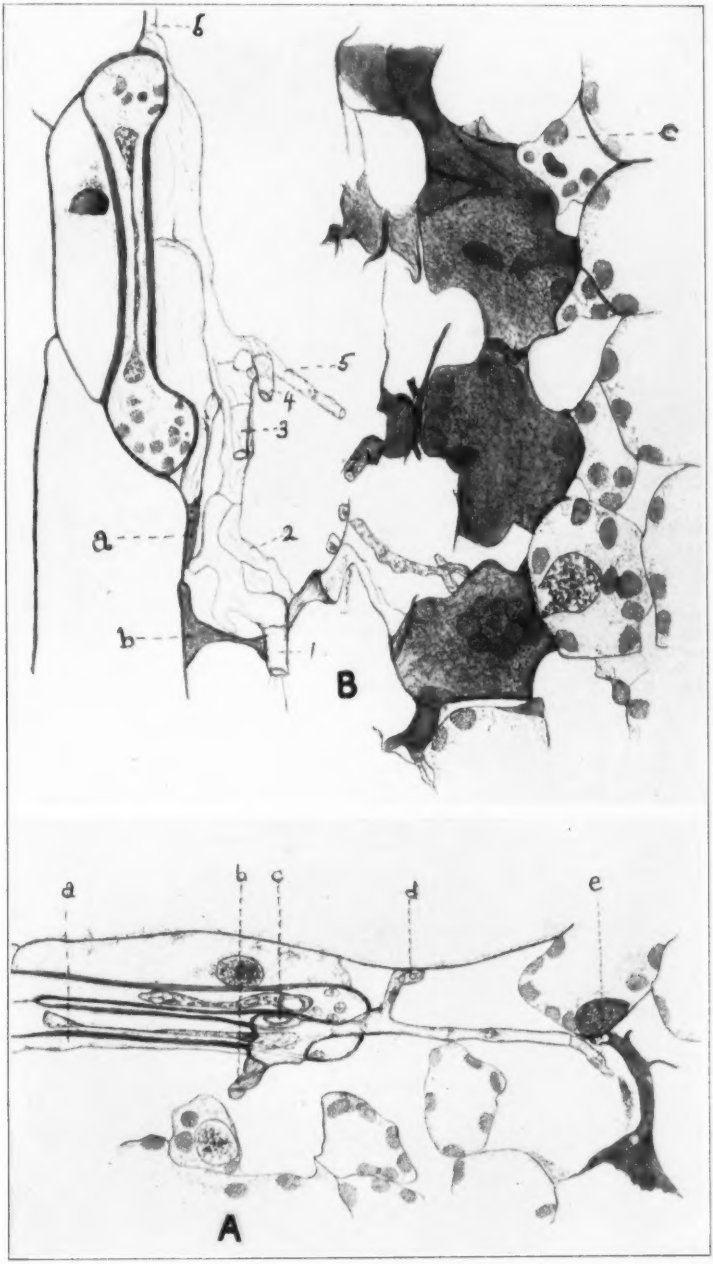
D.—Later stage; one mesophyll cell attacked and its contents disordered. Infecting hypha dead, substomatal vesicle still vigorous.

E.—Oblique section showing remnant of infecting hypha. Contact wall between dead and living host cells thickened.

PLATE 5

A.—Entry of rust through Kanred stoma and the formation of four infecting hyphae at *a*, *b*, *d*, and *e*.

B.—Two substomatal vesicles at one stoma. One produced a single infecting hypha at *a*, killed the host cell at *b*, and died. The second exhausted itself in producing six successive infecting hyphae (No. 1 to 6) and killed a considerable patch of host tissue.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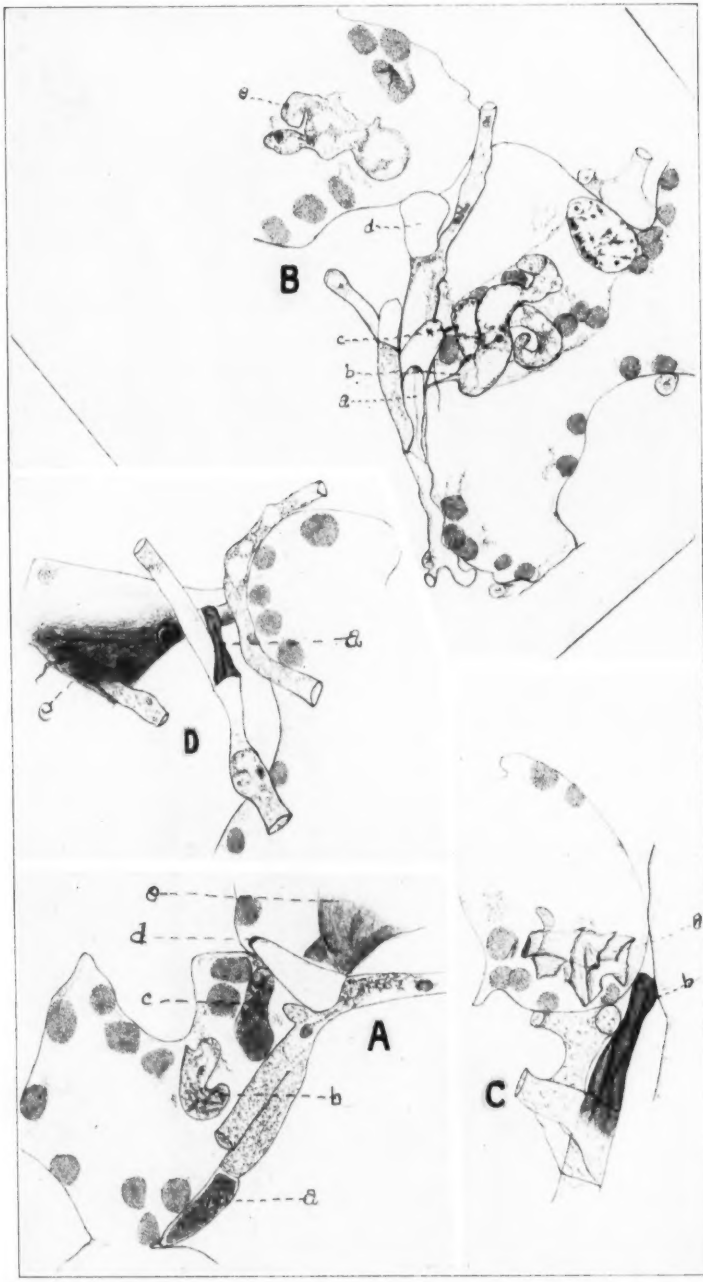
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PLATE 6

A.—Young growth of a small mycelium in Kanred. Haustorium mother cell at *a*, young haustorium without sheath of host cytoplasm at *c*, and host cell killed by the fungus at *e*.

B.—Older portion of same mycelium. Several empty haustorium-mother cells and two large haustoria at *b* and *c*. Host cell contents collapsed about them. Fungus not visibly harmed.

C.—Large haustorium crumpled and dead. The haustorium-mother cell *b* collapsed and the hypha below it empty for a short distance. Host cell normal in appearance.

D.—Later stage. Host cell and hypha both dead.

AFTER-RIPENING AND GERMINATION OF APPLE SEEDS[1]

By GEORGE T. HARRINGTON, *formerly Scientific Assistant,* and BERTHA C. HITE, *Scientific Assistant, Seed-Testing Laboratories, United States Department of Agriculture*

When first matured and in intact condition, apple seeds are wholly incapable of germinating. Furthermore, as will be shown later, they do not acquire the power to germinate under the ordinary conditions for the storage of dry seeds, or under germination conditions at moderate or warm temperatures.

It is the custom of nurserymen to layer apple seeds in sand and put them outdoors over winter. While this practice probably brings about good germination the following spring, Eckerson (*8*)[2] has shown that exposure to freezing temperatures is not at all necessary, as the seeds germinate well after being kept moist for a few months at 5° to 6° C. The present paper deals further with the effect of storage conditions, including the presence or absence of the seed coats and inclusion within or removal from the fruit, on after-ripening and germination.

In the germination tests reported in this paper, usually 25 or 50 seeds, and sometimes 100 or 200 seeds, were used either in single tests or in duplicate tests, according to the number of seeds which were available. The tests were made in Petri dishes with moist blotting paper or absorbent cotton as seed bed.

AFTER-RIPENING AT LOW TEMPERATURES

1. On October 28, 1918, Black Ben Davis apple seeds, which had never been allowed to become air dry, were thoroughly washed, sterilized by treating for two or three minutes with 1 per cent silver nitrate, washed again in running water, which carried chlorids enough to precipitate the silver still remaining on the coats, placed on moist blotting paper in a large Petri dish, and put away in an ice box where the temperature varied between about 5° and about 10° C. By January 15 (two and one-half months) they had begun to germinate in the ice box. Ungerminated seeds from this lot in the ice box were then put to germinate at 20° and at 25°. At each of these temperatures over 50 per cent of the seeds germinated in the next few days. The rest were used for catalase or respiration studies, so that complete germination tests were not obtained.

2. On January 29, 1919, seeds of a mixture of varieties which had been removed from a cider press mash two months earlier and kept in dry storage during the intervening period were washed, sterilized with silver nitrate, and put to germinate at 20° and 30° C. Seeds from the same lot were washed, sterilized, and put away in the ice box to after-ripen.

In the ice box those seeds which did not decay on account of injury in the press after-ripened completely in three or four months, and many germinated during this time, while still in the ice box. At the higher temperatures, however, about 90 per cent of those seeds which did not decay were

[1] Accepted for publication July 2, 1921.
[2] Reference is made by number (italic) to "Literature cited," pp. 160–161.

still dormant after six months, during which time they had received the following treatments: (1) The outer seed coats were removed from all the seeds [3] at the end of 50 days; (2) for the next 68 days those previously at 30° C. were given a daily alternation between 20° and 30°, while those previously at 20° remained at 20°; (3) both lots were then used in a respiration experiment which lasted two months, and in which the temperature was frequently changed, using temperature intervals of 19° to 30°, 30° to 19°, 19° to 10°, 10° to 0°, 0° to 13°, 13° to 30°. Besides these temperature changes the seeds were washed in cold water between each two periods of the respiration experiment, or 20 times in all. None of these treatments had induced after-ripening or germination as had incubation in the ice box.

3. Seeds of the same original lot as the foregoing, which were incubated for 50 days at 20° C. in intact condition, and then 68 days in the ice box with the outer seed coats removed, after-ripened completely during this time in the ice box, so that all germinated in 4 days at 19°. As shown in the preceding paragraph, seeds which were given identical treatment except that they were at 20° while these were in the ice box remained dormant.

Seeds of the same original lot kept in dry storage for one year and then put to germinate at 16° C., 20°, and 23° in intact condition, with outer coats removed and with both coats removed, failed to germinate. With both seed coats removed, all embryos decayed within a week without showing any of the signs of life which were exhibited by living embryos that were not after-ripened.[3]

In the preceding paragraphs, apple seeds have been shown to after-ripen when kept moist in the ice box after three conditions of previous treatment:

(1) Seeds incubated in the ice box soon after removal from the fruit and without previously being allowed to dry out. (2) Seeds stored dry for two months before the ice-box incubation. (3) Seeds stored dry two months and then incubated two months at 20° C. previous to ice-box incubation. In each case concurrent germination tests showed that the seeds had not after-ripened under any other circumstances than storage in moist condition in the ice box. Dry storage previous to the germination test, removal of the outer seed coats, and alteration of temperatures during the germination test all failed to induce germination. Furthermore, attempts to force germination by etherizing the seeds were not successful.

We have found that seeds of another species of Pyrus, which were received from the Federal Horticultural Board of the United States Department of Agriculture, incapable of germinating when received by us, after-ripen in the ice box very much the same as the seeds of our cultivated apples.

AFTER-RIPENING IN THE FRUIT DURING COLD STORAGE

In the cases discussed hitherto, the seeds were removed from the fruits and after-ripened in the ice box under good conditions of moisture supply and aeration, the Petri dish covers being frequently removed to facilitate gaseous exchanges. But this was also true of those seeds which were incubated at higher temperatures, at which they failed to after-ripen.

[3] For description of the seed coats and the effect of their removal see p. 157.

The effect of the low temperature upon after-ripening, as previously reported by Eckerson (8) and now verified by us and as reported by Appleman (*1*), Crocker (*5*), Davis and Rose (*7*), and Davis,[4] and Jones (*13*), for other kinds of seeds, suggested that apple seeds might after-ripen also in cold storage within the intact fruit. Ascherson (*2*), in fact, found a germinated apple seed in an undecayed fruit near the end of June. It seems certain that, in order to have remained sound so late in the year, this apple must have been kept where it was very cool most of the time. At any rate, the seed had become capable of germinating while within the fruit.

Through the kindness of Dr. Brooks and Dr. Cooley of the Office of Fruit Pathology of this Department, material was secured for a study of the germination of seeds from cold-stored apples of several varieties. Table I summarizes the results.

A very small percentage of the seeds of varieties stored at 0° C. until February 5, 1919, and a somewhat higher percentage (not more than 3 per cent) of Newtown Pippin seeds stored at 0° until May 7, 1919, had begun to germinate intracarpially by the time they were taken out of the fruit a few days after taking the apples out of cold storage. When put to germinate at 25°, a few days after removal from the fruits, about one-fifth to one-half of the seeds germinated promptly and vigorously. In every case, however, there was very little germination at 25° after the very incomplete germination of the first few days. In the ice box, on the contrary, germination started rather slowly but progressed gradually until practically complete in about three weeks. The temperature of the ice box occasionally rose considerably above 10° for a short time but was usually between 5° and 10°. At 20° germination was nearly complete in two weeks.

TABLE I.—*Germination of apple seeds after-ripened in cold storage and ice box*[1]

Variety.	Previous storage, successive periods.			Germination.					
				Ice box.		20° C.		25° C.	
	0° C., in the fruit.	Room temperature.	Ice box.[2]	Days.	Per cent.	Days.	Per cent.	Days.	Per cent.
		Days.	*Days.*						
Newtown Pippin.	Oct. 29 to May 7, 190 days.	2	6	12	72	.....	.....	11	56
				25	100				
	Oct. 29 to May 7, 190 days.	7	1	12	72	13	94	11	36
				25	100				
	Nov. 28 to May 7, 160 days.	7	1	12	62	13	98	11	30
				25	94				
York Imperial.	Oct. 1 to Feb. 5, 127 days.	10	4	7	23	.....	.....	7	22
				24	94			24	22
Grimes Golden.	To Feb. 10.....	5	4	7	32	.....	.....	7	45
				24	100			24	50
Jonathan..	Oct. 1 to Feb. 5, 127 days.[3]	10	4	7	7	.....	.....	7	25
				24	93			24	25

[1] *Some seeds had already germinated in the ice box before the beginning of the germination tests. Only those not yet germinated were included in the tests.*

[2] *Most of the time at room temperature the seeds were within the fruits; sometimes dry-stored a few days after taking out. In the ice box the seeds, freed from the fruits, were incubated as for germination.*

[3] *During the last two or three weeks of this time the Jonathan apples were kept at 5° C.*

[4] DAVIS, W. E. PRIMARY, SECONDARY, AND TERTIARY DORMANCY IN THE EMBRYOS OF AMBROSIA TRIFIDA. Paper read at meeting of Amer. Assoc. Adv. Sci., St. Louis, Jan., 1920. Abstract furnished by courtesy of the author. (Not published.)

The germination of Newtown Pippin seeds at 25° C. decreased with increase in the length of time the seeds were kept at room temperature after cold storage in the fruit. Also York Imperial and Jonathan seeds which had been at room temperature for 10 days germinated only half as well at 25° as Grimes Golden seeds which had been at room temperature only 5 days. During the period at room temperature the seeds were either still inclosed in the fruit, or, if removed for a part of the time, were not allowed to become entirely air-dry. While the data at hand are not conclusive, the behavior of these seeds suggests that they were acquiring the condition of secondary dormancy which has been induced in a number of kinds of seeds by keeping the fully germinable seeds under conditions unfavorable for germination. Atwood (*3*), Crocker (*5*), Crocker and Harrington (*6*), and Zade (*23, 24*) have called attention to the assumption of secondary dormancy by various seeds in which the embryo is always capable of germination and dormancy is imposed by coat structures. Davis[5] and Jones (*13*) also have demonstrated the occurrence of this phenomenon in seeds in which, as in the apple seed, the dormant embryo itself is incapable of germination, and in which coats play only a secondary rôle in dormancy.

In January, 1920, seeds from Snow apples, which had been kept in a cold cellar in Vermont until early in January and were then sent by express to Washington, D. C., were put to germinate at 16°, 20°, 23°, and 27° C., and in an ice box where the temperature was about 4° to 5° for a few days and then increased gradually to about 10° at the end of the second week and 12° at the end of the third week. Table II shows their germination.

In the ice box all of the seeds germinated in three weeks, although there was no germination in the first 10 days when the ice box was very cool. The percentage of germination decreased regularly with increase in germination temperature. No seeds germinated after the first 7 days at temperatures above 20° C. and only a small percentage even at 16° C.

TABLE II.—*Germination of Snow apple seeds after-ripened in the fruit in a Vermont cellar. Germination test begun Jan. 14, 1920*

Temperature.	Percentage of germinated seeds removed in—							
	7 days.	10 days.	13 days.	14 days.	16 days.	19 days.	21 days.	Total.
Ice box[1]	0	0	42	36	12	6	4	100
16° C.	52	12	8	0	0	0	2	74
20° C.	48	2	4	2	0	0	0	56
23° C.	46	0	0	0	0	0	0	46
27° C.	12	0	0	0	0	0	0	12

[1] Temperature 4° to 5° C. for about a week, then gradually rising to about 10° at the end of the second week and 12° at the end of the third week.

RELATION OF OXYGEN SUPPLY TO AFTER-RIPENING

The fact that apple seeds after-ripen and sometimes even begin to germinate within the apples while these are still sound raises the question of the relation of oxygen to the processes of after-ripening. Atwood (*3*) has shown that the access of free oxygen in abundant supply is necessary

[5] Davis, W. E., op. cit.

for the after-ripening of wild oats, while Kondo (*18*) has shown that abundant oxygen accelerates the after-ripening of rice, which, when fully after-ripened, will, according to Takahashi (*22*), germinate in entire absence of oxygen. Oxygen has also been shown by Kiessling (*16*) to accelerate after-ripening in cereals under certain conditions, and Hoffman (*12*) has put forth the hypothesis that after-ripening of the cereals consists essentially of a storage of oxygen. Hiltner (*11*) and Kiessling (*17*) have also shown, however, that cereals after-ripen, though sometimes rather slowly, in closed vessels in almost entire absence of oxygen. If free oxygen is essential for after-ripening in this case, the seeds must be capable of using it when present in very low partial pressures, and very small quantities must suffice. Recent and earlier work by one of us (*9*, *10*), as well as Atwood's work on wild oats, indicated that the embryos of the cereals and some other grasses are never essentially dormant, the dormancy of the caryopses being imposed by coat structures which, in the wild oat at least, limit oxygen supply. Crocker (*4*) found a similar condition in Xanthium seeds.

The case is different with apple seeds, in which the embryos are dormant and require time-consuming changes before they can germinate, even if freed from all surrounding structures. But with apple seeds we have found very low respiratory quotients at low temperatures which favor their after-ripening. There is, therefore, in apple seeds a storage of oxygen during after-ripening. Jones (*13*) has shown that the after-ripening of the similarly dormant embryos of sugar maple seeds is retarded, though not prevented, by inclosing them in desiccators, so that oxygen is rapidly replaced by respired carbon dioxid. Recent measurements by Magness (*19*) show that the concentration of oxygen within the core of the apple is not much below that of the surrounding atmosphere. The oxygen supply available to the seeds while inclosed within the fruit cannot, therefore, be considered physiologically deficient for their after-ripening processes, even if these depend upon a much greater abundance of oxygen than seems to be necessary for the after-ripening of sugar maple seeds.

EFFECT OF REMOVAL OF THE SEED COATS

The coverings of the embryos in a mature apple seed consists of: (1) A thick, brown, fibrous outer seed coat with open hilum; (2) a thin, translucent inner seed coat of very dense structure and without openings; and (3) a delicate, whiteish, cellular tissue, somewhat thicker than the inner coat, and apparently endospermous. Layer 3 is closely adherent to layer 2, and it is impossible to remove the two separately, though the embryo is easily bared by removing them together.

Removal of the outer seed coat has no effect on the germination of apple seed previously dry-stored or incubated for a short time at temperatures unfavorable for after-ripening. When the inner seed coats and adhering endosperm tissue are removed from such seeds, baring the embryos, many of the cotyledons enlarge slowly and, if in the light, become green. After a week or two some of the radicles show geotropic bending and slow elongation, but normal growth does not occur.

When one or both of the seed coats are removed from after-ripened seeds germination is accelerated even under conditions under which the seeds would germinate in a few days if the coats were left intact. An example of this is furnished by Newtown Pippin seeds which were put

to germinate at 19° C. after cold storage in the fruit until May 7, 1919, followed by removal from the fruit and incubation in the ice box for about a week. With the outer coats removed 67 per cent germinated in 3 days and 100 per cent in 6 days; with the outer coats left on 26 per cent germinated in 3 days and about 50 per cent in 6 days.

The effect of the removal of the seed coats from seeds which have previously been incubated for a considerable time at temperatures unfavorable for after-ripening, and which are still incapable of germinating in intact condition is of special interst, Seeds of the cider press lot, which had remained dormant under germination conditions for 6 months—4 months with outer coats removed—all germinated in 3 days after removal of the inner seed coats.

With intact York Imperial and Newton Pippin seeds, which had been dormant under germination conditions for more than 5 months and more than 2 months, respectively, the respiratory intensity was increased more than four-fold in 24 hours by removal of the outer seed coats. The inner seed coats were then removed and all of the seeds germinated in the next 2 days. It is evident that these embryos were in a very different condition when freed from the coats than were the embryos of the cider press lot which were freed from the coats soon after putting to germinate 6 months earlier, and which then failed to germinate. Yet as far as ability to germinate with the coats on is concerned they showed no difference. There is here a joint action of the coats and of the condition of the embryo in imposing dormancy, whereas in the dry-stored seeds the same result is produced without the coats. It seems, therefore, that some part of the complex of processes which constitute after-ripening must go on both at the higher temperatures and at the after-ripening temperatures. Apparently other processes, perhaps consisting essentially in the removal of inhibitors to germination (which can also be dissipated or oxidized upon removal of the coats) take place only at the lower temperatures.

In this connection Kidd's work (*14*) on CO_2 and Maze's (*20*, *21*) on acetic aldehyde as inhibitors to germination are of interest. Experiments show that there is a free interchange of oxygen and CO_2 responding in characteristic ways to differences in temperature, in the respiration of dormant apple seeds. This makes it seem unlikely that inhibiting concentrations of CO_2 accumulate within their coats. That some other inhibiting substance is produced at higher temperatures and is held in in by the coats, or else, being initially present, is removed at lower temperatures, seems more plausible; but no work was done to test the validity of such an assumption.

An alternative hypothesis is Kidd and West's conception (*15*) of a mechanical stimulus according to which dormant white mustard embryos are considered as in a state of very delicate equilibrium from which they are aroused to activity by the mechanical shock resulting from removal of the coats, without reference to any causal chemical change.

The inner seed coats are very effective in preventing decay of the embryos during the after-ripening, whereas the thick hard outer seed coats with their open hila are of little value in this respect. This was shown by seeds of the cider press lot, many of which had been so injured that it was impossible to prevent severe decay in the incubator even after carefully discarding all seeds showing visible injury and surface sterilization of the remaining seeds. After 50 days' incubation at 20°, 25°, and

30° C., about 50 per cent of these seeds were wholly decayed, and the rest were entirely obscured by a dense mass of all sorts of micro-organisms. Observations a few days after first putting these seeds into the incubators indicated that in general only those seeds decayed in which the inner coat was injured. At the end of 50 days the outer coats of all the seeds were so softened and decayed that they were easily rubbed off between the thumb and fingers in water. After thorough washing, these seeds, with the outer coats removed but the inner coats intact, were returned to the incubators where almost all of them remained in good condition during the following 4 months, although during a part of that time they were on moist blotters throughly infected with organisms of decay.

SUMMARY

(1) Apple seeds, when taken from the apples at their maturity, are incapable of germination. Etherization or the use of alternating temperatures does not bring about germination.

(2) The dormancy is resident in the embryo. Naked embryos fail to germinate normally.

(3) Apple seeds acquire the power to germinate—that is, they after-ripen, in a few months when kept moist at a temperature between 5° and 10° C. They also after-ripen within the fruit in commercial cold storage (0° C) or in a cold cellar. They do not after-ripen in dry storage or when kept moist at 20° C. or at a higher temperature.

(4) The relation of oxygen to after-ripening was not determined, but apparently a good supply of oxygen is always present within the core of the apples when they are kept at low temperatures.

(5) After-ripened seeds will germinate completely in a few weeks in an ice box if the temperature is not too low. They germinate fairly well at 20° C., but not as well at 25°. The optimum temperature for their germination seems to lie somewhere between 10° and 20° and to vary according to the condition of the seed or possibly according to the variety of apple.

(6) There seems to be a tendency for the after-ripened seeds to go into a state of secondary dormancy when kept under conditions which prevent their germination.

(7) The commercial practice of layering apple seeds out of doors over winter is not necessary in order to bring about their complete after-ripening and germination.

(8) Removal of the outer seed coat has no apparent effect on completely dormant apple seeds. Removal of both seed coats causes some of the dormant embryos to make feeble growth, but these do not produce normal seedlings.

(9) Removal of the outer coat or of both coats accelerates the germination of after-ripened seeds.

(10) Removal of the coats from seeds which have been incubated for a long time under germination conditions, but at a temperature too high for complete after-ripening, may induce prompt and vigorous germination. Some phases of after-ripening must therefore take place at these higher temperatures, while others are dependent upon a lower temperature, or upon the removal of the coats.

(11) The inner seed coat is very efficient in preventing decay of the seeds, but the outer seed coat, with its open hilum, is of little use in this respect.

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MISCELLANEOUS TESTS OF CARBON TETRACHLORID AS AN ANTHELMINTIC [1]

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On the basis of what is known about it at present, carbon tetrachlorid (CCl_4) promises to be an uncommonly valuable addition to the list of anthelmintics, having a high efficacy against worms of several sorts, an unusually deadly effect on bloodsucking worms, and a large margin of safety, so far as it has been tested, for most of the eight species of host animals used. It apparently offers a solution for several problems in removing worms which no anthelmintic has been heretofore known to solve satisfactorily. For these reasons it is a drug which deserves to be brought to the attention of veterinarians and of medical men in general.

HISTORICAL

More than half a century ago carbon tetrachlorid was used to a slight extent as an anesthetic and analgesic. The literature on this subject has been briefly summarized by Hall (*13*) [2] in a recent paper. For half a century this drug has been disregarded in medicine, though it has received more or less attention in other fields and is much used as a fire extinguisher, cleaner, insecticide, and solvent for fats, gums, etc. During 1921 Hall recommended it as an anthelmintic. In a paper by Hall (*13*) it was reported as 100 per cent effective in removing hookworms from dogs when properly administered in suitable doses. In another paper by Hall (*15*), experiments were reported on carbon tetrachlorid as a drug for the removal of bots and worms from horses, the drug being as effective as oil of chenopodium in removing Strongylus but somewhat less effective than carbon bisulphid (CS_2) in removing bots. In a third paper by Hall (*16*), carbon tetrachlorid was brought to the attention of the medical profession as being worthy of trial against human hookworms. In the same paper some toxicity tests of this drug on monkeys are reported, the tests indicating that these animals will tolerate rather large doses without bad effects; and one test is reported in which the writer took a dose of 3 cc. of carbon tetrachlorid without ill effects of any sort. In this paper we have summarized the published findings and added the results of our experiments.

TESTS ON TURKEYS

In the following experiments with carbon tetrachlorid on turkeys, four mature birds were used. The drug was given by means of a catheter passed down the esophagus. It is very easy to administer drugs to poultry in this way.

Turkey No. 15.—Weight 6.33 kilos; dose rate 1 cc. per kilo; no worms passed in 4 days; feces scant but no ill effects noted.

[1] Accepted for publication Nov. 3, 1922. Read in abstract at the tenth meeting of the Illinois State Veterinary Medical Association at Chicago, Dec. 2, 1921.

[2] Reference is made by number (italic) to "Literature cited," p. 191–192.

Turkey No. 16.—Weight 5.68 kilos; dose rate 0.5 cc. per kilo; no worms passed in 4 days and no ill effects noted.

Turkey No. 17.—Weight 6.5 kilos; dose rate 0.5 cc. per kilo; no worms passed in 4 days and no ill effects noted.

Turkey No. 18.—Weight 5.5 kilos; dose rate 1 cc. per kilo; no worms passed in 4 days and no ill effects noted.

The turkeys used were several years old and, as is often true of mature animals, were very lightly infested with parasites of the digestive tract. The only bird to show worm eggs in the feces at the beginning of the experiment was No. 15, which showed a few Capillaria present. The findings from these tests show nothing as to the anthelmintic efficacy of carbon tetrachlorid since no post-mortem examinations were made, but they show that turkeys will tolerate doses of carbon tetrachlorid at the rate of 0.5 to 1 cc. per kilo of weight. From the following experiments on chickens it appears that turkeys will probably tolerate very large doses of carbon tetrachlorid.

TESTS ON CHICKENS

Carbon tetrachlorid was administered to 13 chickens by means of a catheter passed down the esophagus, the tests being made to determine anthelmintic efficacy and toxicity. The experiments were as follows:

Bird No. 1.—Weight 1.505 kilos; dose rate 1 cc. per kilo; no worms passed in 3 days; post mortem, 1 spirurid in gizzard and numerous Hymenolepis in small intestine.

Bird No. 2.—Weight 1.45 kilos; dose rate 1 cc. per kilo; no worms passed in 3 days; post mortem, 5 *Heterakis papillosa* and 1 Capillaria in ceca.

Bird No. 3.—Weight 1.29 kilos; dose rate 1 cc. per kilo; passed 34 *Heterakis papillosa* in 3 days; post mortem, 72 Tetrameres in proventriculus, 6 spirurids in gizzard, and 6 *H. papillosa* in ceca.

Bird No. 4.—Weight 1.175 kilos; dose rate 1 cc. per kilo; no worms passed in 3 days; post mortem, 13 *Ascaridia perspicillum* and numerous tapeworms in small intestine.

Bird No. 5.—Weight 1.285 kilos; dose rate 2 cc. per kilo; passed 1 *Ascaridia perspicillum* in 4 days; post mortem, few *Gongylonema ingluvicola* in crop, few Capillaria and Hymenolepis in small intestine, and 5 *Heterakis papillosa* in cecum and large intestine.

Bird No. 6.—Weight 1.07 kilos; dose rate 2 cc. per kilo; no worms passed in 4 days; post mortem, some *Davainea proglottina* in small intestine.

Bird No. 7.—Weight 1.25 kilos; dose rate 2 cc. per kilo; no worms passed in 4 days; post mortem, 1 spirurid in gizzard, 15 *Heterakis papillosa* and 1 Capillaria in cecum.

Bird No. 8.—Weight 1.385 per kilos; dose rate 2 cc. per kilo; no worms passed in 4 days; post mortem, few Tetrameres in proventriculus and few tapeworms in small intestine.

Bird No. 9.—Weight 1.255 kilos; dose rate 2 cc. per kilo; no worms passed in 4 days; second dose at rate of 5 cc. per kilo, passed 2 *Ascaridia perspicillum* in 4 days; third dose at rate of 6 cc. per kilo, no worms passed in 3 days; fourth dose (7 days after third dose) at rate of 12 cc. per kilo, no worms passed in 3 days; post mortem (12 days after fourth dose), 25 tapeworms in small intestine.

Bird No. 10.—Weight 1.51 kilos, dose rate 2 cc. per kilo, no worms passed in 4 days; second dose at rate of 5 cc. per kilo, no worms passed in 4 days; third dose at rate of 7 cc. per kilo, no worms passed in 3 days; fourth dose (7 days after third dose) at rate of 15 cc. per kilo, no worms passed in 3 days; post mortem (12 days after fourth dose), no worms present.

Bird No. 11.—Weight 1.33 kilos; dose rate 3 cc. per kilo, passed 1 *Heterakis papillosa* in 4 days; second dose at rate of 6 cc. per kilo, no worms passed in 4 days; third dose at rate of 8 cc. per kilo, no worms passed in 3 days; fourth dose (7 days after third dose) at rate of 18 cc. per kilo, no worms passed in 3 days; post mortem (12 days after fourth dose), 1 tapeworm in small intestine.

Bird No. 12.—Weight 1.265 kilos; dose rate 3 cc. per kilo, no worms passed in 4 days; second dose (8 days after first dose) at rate of 10 cc. per kilo, no worms passed in 3 days; third dose (7 days after second dose) at rate of 20 cc. per kilo, no worms passed in 3 days; post mortem (12 days after third dose), no worms present.

Bird No. 13.—Weight 1.29 kilos; dose rate 4 cc. per kilo; passed 2 *Ascaridia perspicillum* and 5 *Heterakis papillosa* in 4 days; post mortem, no worms present.

The most striking feature of these experiments is the tolerance of chickens for carbon tetrachlorid. The largest single dose administered (bird No. 12) was at the rate of 20 cc. per kilo of weight of animal, which is approximately 67 times the therapeutic dose rate for removing hookworms from dogs and foxes. In a period of 15 days this bird was given carbon tetrachlorid in quantities equivalent to a total at the rate of 33 cc. per kilo, or 110 times the therapeutic dose for dogs. Bird No. 11 was given carbon tetrachlorid in a period of 15 days in quantities equivalent to a total at the rate of 35 cc. per kilo, or almost 117 times the therapeutic dose for dogs; this bird was given one dose of carbon tetrachlorid at the rate of 18 cc. per kilo, or 60 times the therapeutic dose for dogs. None of the birds showed signs of discomfort or injury as a result of the treatment, and on post-mortem examination they appeared normal except for some evidence of inflammation in the small intestine of the birds given repeated large doses (birds No. 9, 10, 11, and 12). The survival of these birds and their good condition indicated that no serious injury had been caused by the drug.

As regards efficacy, no worms of the superfamily Spiruroidea—those present in these birds being forms which live in the tissues along the digestive tract, including *Cheilospirura hamulosa* in the wall of the gizzard, *Tetrameres* sp. in the proventriculus, and *Gongylonema ingluvicola* in the wall of the crop—were found in the droppings after treatment. Apparently, the treatment was ineffective in removing these worms. However, it should be noted that none of these worms were found post mortem in the five birds which received large and repeated doses, and there is a possibility that flooding the digestive tract with large quantities of carbon tetrachlorid may have resulted in the death of the worms, with the failure to find them in the droppings owing to the worms having been destroyed by digestion. These worms are situated in the anterior portion of the digestive tract and it seems reasonable to suppose that they would be destroyed and digested, at least to an extent which would make the fragments unrecognizable in the droppings. It would appear quite unlikely that worms would pass through the gizzard without being fragmented.

The drug apparently was entirely ineffective in removing hairworms of the genus *Capillaria* in cases where it was given in single doses of 1 or 2 cc. per kilo, but the failure to find these worms post mortem in the five birds given large and repeated doses suggests that these doses may have killed the worms although the dead worms were not found in the feces, owing to their small size and the difficulty of finding them.

As regards the efficacy of the drug against *Ascaridia perspicillum*, it failed to remove any of these worms from one bird when given at the rate of 1 cc. per kilo but removed all the worms present in three cases when given at the rate of 2, 4, and 5 cc. per kilo.

As regards its efficacy against *Heterakis papillosa*, carbon tetrachlorid in doses of 1 or 2 cc. per kilo failed to remove any of a total of 25 worms present in three birds. In another case the drug, at a dose rate of 1 cc. per kilo, removed 34 of 40 worms present, or 85 per cent. In the case of the five birds given large and repeated doses, no worms were found post mortem, this fact suggesting that some worms may have been removed although not found in the droppings. These findings suggest that the drug may be effective when it actually enters the ceca and gets in contact with the worms present. They also suggest that the entrance of the drug might be insured by the use of large or of repeated doses. This would appear to be feasible in conditions where the presence of these worms warranted it, inasmuch as the drug appears to be quite safe and well tolerated in large doses.

The drug was quite ineffective against tapeworms in chickens, as in other animals.

An additional test of the effect of carbon tetrachlorid on chickens (Rhode Island Reds) was conducted as follows: 16 chickens, of which 8 were 1 year old and 8 were 2 years old, weighing on an average about 5 pounds each, were each given 1.5 fluid drams, about 5.5 cc., of carbon tetrachlorid by catheter to the crop, the birds not being fasted. The dose rate was about 2.27 cc. per kilo. The egg production for the 10 days previous to treatment averaged 9.6 eggs a day; for the day of treatment, May 14, it averaged 8 eggs; and for the 10 days following treatment, it averaged 10.8 eggs a day. It is evident from these results that the treatment had no deleterious effect on egg production. On the other hand, it could not be assumed from one test on 16 chickens that the increase in egg production, amounting to 1.2 eggs a day, was correlated with the administration of carbon tetrachlorid.

Hall and Shillinger[3] report some experiments with drugs injected by rectum into chickens for the removal of the cecum worm, *Heterakis papillosa*, the drugs used including carbon tetrachlorid. Six chickens, weighing about 1.5 pounds each, were injected with this drug. The quantities given and the results obtained in removing *H. papillosa* are as follows:

No. 14; 10 cc.; passed 79 worms; post mortem, no worms; efficacy, 100 per cent.

No. 15; 10 cc.; passed 2 worms; post mortem, no worms; efficacy, 100 per cent.

No. 16; 5 cc.; passed 1 worm; post mortem, no worms; efficacy, 100 per cent.

[3] HALL, Maurice C., and SHILLINGER, Jacob E. THE REMOVAL OF HETERAKIDS FROM THE CECA OF CHICKENS BY RECTAL INJECTIONS OF ANTHELMINTICS. *In* Jour. Amer. Vet. Med. Assoc. (In press.)

No. 17; 5 cc.; passed no worms; post mortem, 10 worms; efficacy, 0 per cent.

No. 18; 3 cc.; passed 24 worms; post mortem, 70 worms; efficacy, 35.5 per cent.

No. 19; 2 cc.; passed 41 worms; post mortem, no worms; efficacy, 100 per cent.

Total worms passed, 147; total remaining post mortem, 80; efficacy, 65 per cent.

All the worms were removed from 4, or 66.6 per cent, of the 6 birds listed above. While the smallest dose, 2 cc., was as effective as the largest dose, the removal of all the worms present seems more certain where the larger doses are used. The most satisfactory treatment for the removal of these worms was found to be the rectal injection of 0.1 cc. of oil of chenopodium in 5 cc. of a bland oil (cottonseed oil). The dose for birds weighing 3 pounds or more would be double this, or 0.2 cc. in 10 cc. of bland oil. The injections were made with an infant's-size rectal syringe of hard rubber, the tip of the syringe being passed along the floor of the cloaca and the fluid injected slowly.

TESTS ON RABBITS

The following tests of the toxicity of carbon tetrachlorid administered by stomach tube to rabbits were conducted:

Rabbit No. 7213.—Weight 2.055 kilos; dose 5 cc. per kilo; animal off feed for a few days, but no other ill effects noted; discharged from experiment eight weeks later.

Rabbit No. 7214.—Weight 1.755 kilos; dose 10 cc. per kilo; animal stupid the following day, dull and weak the second day, found dead the morning of the third day; post mortem, stomach inflamed and slightly hemorrhagic in cardia, small and large intestines showed areas of submucous hemorrhages and some hemorrhage to lumen, liver pale, heart muscle injected, anterior lobes of lungs congested and some marginal edema present, peritoneal fluid excessive and slightly sanguineous, bladder apparently normal.

Rabbit No. 7215.—Weight 1.97 kilos; dose 15 cc. per kilo; animal died the following day; post mortem, stomach inflamed showing hemorrhagic areas, small intestine with areas of inflammation and ecchymoses, large intestine congested, carbon tetrachlorid recovered from stomach and large and small intestines, heart vessels congested, lungs showed more or less extensive capillary hemorrhage, vessels of urinary bladder congested.

Rabbit No. 7216.—Weight 1.65 kilos; dose 20 cc. per kilo; animal died the following day; post mortem, evidence of diarrhea, gastric cardia showed hemorrhagic eroded areas, ecchymoses in small intestine, large intestine congested, carbon tetrachlorid recovered from stomach and large and small intestines, heart vessels congested, urinary bladder empty and its blood vessels injected.

These tests indicate that rabbits will tolerate doses of carbon tetrachlorid at the rate of 5 cc. per kilo, but will die in the course of 2 days after a dose at the rate of 10 cc. per kilo and within 24 hours after a dose at the rate of 15 or 20 cc. per kilo. The minimum lethal dose rate is therefore between 5 and 10 cc. per kilo, apparently close to 10 cc. per kilo. The experiment indicates that carbon tetrachlorid passes through the digestive tract unabsorbed in most part, but that in large doses

enough may be absorbed to cause death. The dose which was tolerated, at the rate of 5 cc. per kilo, would give a safety factor of almost 17 on the basis of the therapeutic dose for dogs—namely, 0.3 cc. per kilo. This is a distinctly lower safety factor than that for this drug in the case of dogs, but it is nevertheless a high safety factor for drugs in general. A very casual determination by water displacement of the gastric capacity of a rabbit weighing 7 pounds (a little over 3 kilos) showed it to be about 200 cc. Ten cc. per kilo being considered as the minimum lethal dose, the minimum lethal dose for this rabbit would be over 60 cc., or more than 30 per cent of the gastric capacity. A rabbit weighing 1.97 kilos had a gastric capacity of 125 cc. of which the minimum lethal dose would equal almost 16 per cent, and a rabbit weighing 1.65 kilos had a gastric capacity of 120 cc. of which the minimum lethal dose would equal almost 14 per cent.

TESTS ON DOGS

In a paper by Hall (*13*), already referred to, tests were reported of the use of carbon tetrachlorid on 30 dogs. These tests showed that when administered in hard capsules in doses of 0.3 cc. per kilo of live weight—a dose of 3 cc. for a dog of average weight, 10 kilos (22 pounds)—this drug would remove all the hookworms present. Apparently, no purgation is necessary and the drug itself is not constipating. Equally good results were obtained by the use of a solution containing 10 grains of thymol to 1 cc. of carbon tetrachlorid, the solution being given at the same dose rate as the carbon tetrachlorid alone, 0.3 cc. per kilo. One hundred per cent efficacy was also obtained by the use of a solution containing 3 parts by volume of carbon tetrachlorid and 1 part of oil of chenopodium, the solution being given at the same dose rate as the carbon tetrachlorid alone. The removal of all hookworms present by carbon tetrachlorid, or mixtures containing this drug, from a series of infested dogs is a result which it has been impossible to obtain in scores of experiments with chenopodium, chloroform, thymol, and other drugs. It apparently solves the problem of treatment for hookworm disease in dogs. It has been favorably reported on by Smyth (*29*), and we have had favorable reports in correspondence from and in conversation with a number of veterinarians in various parts of the United States. Brumley in 1922 stated: "I wish to report that carbon tetrachlorid has given us excellent results in removing hookworms from dogs. Its use has demonstrated its superiority over any of the other anthelmintics used to date." Dr. D. E. Buckingham, of Washington, D. C., tells us that he has given it to more than 500 dogs and cats with no bad results, even in the case of pups 2 weeks old or kittens 3 weeks old.

When administered in hard capsules at the dose rate stated above, carbon tetrachlorid removed not only all the hookworms, but also all the ascarids present. Other experiments at different dose rates indicate that this drug is probably not so effective against ascarids as is oil of chenopodium, but it appears to be only second to this highly effective drug in removing these worms.

Carbon tetrachlorid is substantially as effective in removing whipworms as is any other good anthelmintic. In a general way one may say that almost any anthelmintic will remove whipworms if it comes in contact with them, but the establishment of such a contact is usually a matter of accident. If an anthelmintic enters the cecum after passing the ileocecal valve it will probably remove the whipworms with which

it comes in contact. If the anthelmintic passes from the ileocecal valve down the colon, it will not come in contact with the whipworms in the cecum, and except in very heavy infestations the whipworms present are usually found clustered near the tip of the cecum. In the experiments on dogs it was reported that when carbon tetrachlorid was administered in capsules at a dose rate of 0.1 or 0.2 cc. per kilo it removed no whipworms, at a dose rate of 0.3 cc per kilo it removed less than 10 per cent of the whipworms, and at a dose rate of 1 cc. per kilo it removed 100 per cent of the whipworms present in one dog. These results suggest that the problem of removing whipworms by insuring the entrance of the drug into the cecum might be solved in either of two ways. One of them is to give repeated small doses of some anthelmintic which is not a gastro-intestinal irritant, such as santonin, over a period of a number of days in order that the entry of the drug into the cecum be insured by increasing the number of its chances to enter the cecum. This is a method which has been tested and proposed by Hall (*9, 12*). Another solution of the problem would be to employ a drug which could be used in such large quantities that its entrance into the cecum would be insured because of the large quantity used. Carbon tetrachlorid might be such a drug, though this remains to be proved. However, it will be noted that as the dose rate for this drug was raised, the efficacy increased from 0 to 100 per cent, the latter result being attained with a dose of 1 cc. per kilo. Tests of toxicity were reported by Hall (*12*), showing that dogs would survive a dose of 1.5 cc. per kilo without showing symptoms of injury or any evident lesions post mortem.

We have carried on additional toxicity tests of this drug on dogs as follows: Three dogs (No. 436, 425, and 426) weighing, respectively, 20.5 pounds (about 9 kilos), 21.5 pounds (about 10 kilos), and 39.5 pounds (about 18 kilos) were each given 10 cc. of carbon tetrachlorid in capsules. Seven days later they were given, respectively, 30, 50, and 60 cc. of this drug by catheter to the stomach, a dose rate of 3.33 to 5 cc. per kilo. Thirty-nine days later the first two of these dogs (No. 436 and 425) were given, respectively, 91 to 60 cc. by catheter to the stomach. At this time dog No. 436 weighed 13 kilos, the dose rate being 7 cc. per kilo. One week later, dog No. 425, previously given 60 cc. of carbon tetrachlorid, was given 156 cc., or 12 cc. per kilo; and another dog, No. 424, weighing 20 kilos, was given 240 cc., or 12 cc. per kilo. A little less than two months later, dog No. 424 was given 320 cc., or 16 cc. per kilo. These animals showed no bad effects immediately after treatment or subsequently. Since no bad effects were observed, the highest dose rate, 16 cc. per kilo, gives a safety factor of over 53 for the therapeutic dose of 0.3 cc. per kilo. The two doses being considered, dog No. 424 received in less than two months 560 cc., or 28 cc. per kilo, or more than 93 times the therapeutic dose. At a single dose this dog received over 53 times the therapeutic dose. These figures indicate that carbon tetrachlorid is uncommonly safe. The therapeutic dose of oil of chenopodium for the removal of all ascarids present is 0.1 cc. per kilo. The minimum lethal dose is 0.5 cc. per kilo. This gives a safety factor of 5. So far we have failed to find the minimum lethal dose rate for carbon tetrachlorid, since our dogs have survived what we regarded as dangerously high doses; but the present ascertained safety factor is over 53, or more than ten times as great as that of oil of chenopodium as an effective drug for ascarids compared with that of car-

bon tetrachlorid as an effective drug for hookworms. This bears out the supposition that it may prove feasible and safe to flood the digestive tract of dogs with sufficient carbon tetrachlorid to kill whipworms. A safety factor of 53 is a very high safety factor for drugs. According to Hall and Wigdor (*21*) the gastric capacity of the average dog is about 1 liter or a little less. At a dose rate of 16 cc. per kilo, a dog of average weight (10 kilos) could take with safety 160 cc., or about one-sixth (16 per cent) of its stomach capacity of carbon tetrachlorid.

In a paper by Hall (*13*) it is noted that this drug is not of value in removing tapeworms of the genus *Dipylidium*, and probably will not be valuable in removing any tapeworms.

We have carried on a few tests to determine the efficacy of carbon tetrachlorid when administered in soft capsules. Theoretically, the drug should be as effective when given in soft capsules as when given in hard capsules, but since some chemicals harden soft gelatine capsules and in time harden them to the point where they will pass the digestive tract unopened, it seems advisable to determine something definite in regard to the possible effects of carbon tetrachlorid on gelatine and the efficacy of the drug when administered in soft capsules. In the following tests the capsules used by us were from two lots, one less than 3 months old and the other over 4 months old but less than a year old, the date of manufacture not being definitely known to us.

Dog No. 488.—Weight 13.5 pounds (approximately 6 kilos); given 1.5–cc. soft capsule; no worms passed; post mortem, fourth day, 14 hookworms, 46 whipworms, 31 *Dipylidium caninum;* efficacy, 0 per cent against hookworms, whipworms, and tapeworms.

Dog No. 489.—Weight 19 pounds (approximately 9 kilos); given one 3–cc. soft capsule; first day, 16 ascarids, 1 whipworm; second day, 7 whipworms; third day, 2 whipworms; post mortem, fourth day, 28 whipworms, 2 *Dipylidium caninum;* efficacy 100 per cent against ascarids, 26 per cent against whipworms, 0 per cent against tapeworms.

Dog No. 491.—Weight 24 pounds (approximately 11 kilos); given one 3–cc. soft capsule; second day, 1 whipworm; fourth day, 2 hookworms, 1 whipworm; post mortem, fourth day, 74 whipworms; efficacy, 100 per cent against hookworms, 2.6 per cent against whipworms.

Dog No. 492.—Weight 23 pounds (approximately 10.5 kilos) given one 3–cc. soft capsule; first day, 2 ascarids; post mortem, fourth day, 10 whipworms; efficacy, 100 per cent against ascarids, 0 per cent against whipworms.

In the foregoing tests the doses are approximately the therapeutic doses for the weights of the animals. The drug was 100 per cent effective against ascarids in the case of the two infested animals, 7 per cent effective against whipworms, and 0 per cent effective against tapeworms. It was 100 per cent effective against hookworms in the case of one dog and 0 per cent effective in the case of another. These findings must be considered in the light of the fact that all four dogs showed hookworm eggs on preliminary fecal examination. It appears, therefore, that there is some likelihood that all the feces passed were not collected by the attendant and that the other two dogs may have had all their hookworms removed by the treatment. Ignoring these doubtful cases, we may say that carbon tetrachlorid given in soft capsules in approximately the therapeutic dose of 0.3 cc. per kilo may remove all the hookworms present or may leave many or all of them. The dependability

of the drug appears to be lessened by the use of the soft capsule, but the number of tests here are entirely too small to warrant a positive statement. However, it may be pointed out that Hall (*10, 11*) did not find chloroform administered in soft gelatine capsules so effective as when administered in hard capsules or in castor oil. It seemed possible that chloroform exerted a hardening effect on the gelatine, preventing the capsules from opening in time to come in contact with the worms. This same possibility may be true of carbon tetrachlorid, which is closely related to chloroform.

In connection with reiterated recommendations that only the chemically pure carbon tetrachlorid be used, it is of interest to note that Dr. W. J. Ratigan has reported in correspondence the deaths of two dogs treated for worms by their owners under circumstances indicating that commercial carbon tetrachlorid was used.

TESTS ON CATS

Tests of carbon tetrachlorid showed that a dose rate of 0.3 cc. per kilo was well tolerated by cats. We have already noted that Doctor Buckingham reports favorably on the use of carbon tetrachlorid in the case of these animals. Dr. O. V. Brumley, of the Ohio State University, writes in regard to it under date of April 12, 1922: "It has been a great help, particularly in the treatment of hookworm in dogs and cats."

In order to ascertain the minimum lethal dose of carbon tetrachlorid for cats we carried out the following experiments:

Cat No. 3.—Three-months-old kitten, weighing 890 gm. Given 10 cc. of carbon tetrachlorid, a dose rate of slightly over 11.23 cc. per kilo, by stomach tube, a catheter being used as a tube. Treatment was given at 8.30 a. m. Within a half hour the animal vomited some food and 2 immature ascarids. It refused to eat and appeared stupid. The next morning it was found dead. There was a considerable quantity of carbon tetrachlorid in the posterior third of the small intestine; the intestine was empty anterior to this but contained some ingesta in the posterior portion. The stomach and intestine showed very little inflammation. No worms were found post mortem. This animal was thin and in poor condition when treated.

Cat No. 4.—Three-months-old kitten, weighing 1.54 kilos. Given 15 cc. of carbon tetrachlorid, a dose rate of 9.74 cc. per kilo, by stomach tube at 8.30 a. m. Within a half hour the animal vomited some food and 1 mature ascarid. The animal became weaker that day and the next day, dying the second night after treatment. The organs appeared normal on macroscopic examination except for a considerable quantity of mucous material in the small intestine. No worms were found post mortem.

Cat No. 5.—Six-weeks-old kitten, weighing 675 gm. Given 3 cc. of carbon tetrachlorid by stomach tube, a dose rate of slightly over 2 cc. per kilo. During the second hour after treatment the cat vomited 2 ascarids and during the night it passed 6 ascarids. The cat was very sick for several hours, showed improvement in 6 hours, and was apparently normal in 2 days, continuing in good health for 2 weeks, at the end of which time it was given a larger dose.

Cat. No. 6.—Six-weeks-old kitten, weighing 700 gm. Given 4 cc. of carbon tetrachlorid by stomach tube, a dose rate of 2.8 cc. per kilo. Within an hour the cat vomited 9 ascarids and during the night it passed

2 ascarids. The subsequent history was the same as that for No. 5, but the symptoms were less severe following a larger dose (to be reported below).

Cat No. 7.—Six-weeks-old kitten, weighing 960 gm. Given 4.8 cc. of carbon tetrachlorid by stomach tube, a dose rate of 5 cc. per kilo. Within an hour the cat vomited 3 ascarids and during the night it passed 14 ascarids. The subsequent history was the same as for No. 5 and 6, but the symptoms were more severe following a larger dose (to be reported below).

Two weeks after these animals (No. 5, 6, and 7) had been given the foregoing treatments, they, now 2 months old, were given larger doses of carbon tetrachlorid as follows:

Cat No. 5.—Weight 620 gm. Given 3.72 cc. of carbon tetrachlorid by stomach tube, a dose rate of 6 cc. per kilo. The animal vomited within a half hour after treatment but did not seem greatly affected. It drank milk 3 or 4 hours after treatment and seemed quite normal the following day.

Cat No. 6.—Weight 740 gm. Given 5.18 cc. of carbon tetrachlorid by stomach tube, a dose rate of 7 cc. per kilo. The animal did not vomit after treatment and did not seem greatly affected by it. It drank milk 3 or 4 hours after treatment and seemed quite normal the following day.

Cat No. 7.—Weight 940 gm. Given 7.52 cc. of carbon tetrachlorid by stomach tube, a dose rate of 8 cc. per kilo. This animal showed profuse salivation after treatment and pronounced evidence of intoxication. There was evident improvement in its condition in the course of 3 or 4 hours, and it seemed quite normal the following day.

So far as conclusions can be safely drawn from these experiments, it is evident that carbon tetrachlorid has a wide margin of safety between the therapeutic dose of 0.3 cc. per kilo and the minimum lethal dose. Cats No. 3 and 4 were not in very good physical condition, and it is possible that stronger animals would have survived the doses which killed these animals. At any rate, the survival of animals given 8 cc. per kilo gives an indicated safety factor of almost 27, which is considered high. It indicates that members of the Felidae, as well as of the Canidae, are highly tolerant of carbon tetrachlorid.

In connection with the effects of carbon tetrachlorid taken in milk by one of the writers and discussed later in this paper, the drug was given as follows to cats No. 6 and 7 five days after they were given the second dose as reported in the preceding tests:

Cat No. 6.—Given 3.7 cc., a dose rate of 5 cc. per kilo, in 5 cc. of rich milk. The animal did not vomit, and showed no ill effects.

Cat No. 7.—Given 3.7 cc., a dose rate of almost 4 cc. per kilo, in 5 cc. of olive oil. The animal did not vomit, and showed no ill effects.

The use of milk and oil did not result in an evident increase of toxicity.

The same day on which these cats were treated the third time, cat No. 5 was killed and examined post mortem. There were no worms present. The liver was light-colored.

TEST ON FOXES

The writers have carried on no experiments with carbon tetrachlorid for removing worms from foxes, but a number of reports of such experiments, in conversation and in correspondence as well as in published papers, have come to their attention. So far these reports have been

quite favorable, both as regards the efficacy of the drug against hookworms and as regards its safety. This is a matter of considerable interest and importance, since hookworms, mostly *Uncinaria stenocephala*, are very common in fur foxes raised in captivity, and because of the value of these animals, some of them being worth $900 to $2,000, or more, a pair, the loss of an individual animal is a serious matter. One fox raiser states in correspondence that in the last two years he has lost at least $50,000, mostly because of hookworms. This man has reported some interesting experiments on the administration of carbon tetrachlorid to foxes. In two cases a capsule containing 1.55 cc. of carbon tetrachlorid was pushed into the windpipe, causing death in 1 to 2 minutes. This, of course, is a matter of technic.

In administering capsules to hundreds of dogs we have never pushed one into the windpipe. However, it is difficult to avoid this in foxes, since the capsules are given to adult foxes with a balling gun, a much less sensitive instrument than the fingers. Doctor Hanson tells us that capsules may be administered with the fingers to fox pups up to 6 weeks old. This would appear to be the safest and best mode of administration for fox pups. The fox raiser referred to above gave to 3 foxes, weighing about 15 pounds each, doses of about 18.6 cc. of carbon tetrachlorid by drench, a dose rate of approximately 2.7 cc. per kilo. All the animals showed convulsions, probably as a result of inhalation intoxication, and 1 of them died, the other 2 recovering. A report on these cases has been published by Hall (*17*).

Jeffreys (*24*) recommended for dosing pups, the use of ordinary syringe rubber tubing 5 inches long with an outside diameter of $\frac{11}{32}$ inch and a bore of $\frac{1}{4}$ inch, the tip to be rounded with sandpaper. This is fitted with a pliable plunger with a button in the end outside of the tube and with the other end blunt. The mouth of the fox is held open with a speculum, and the end of the tube is oiled and inserted into the upper end of the esophagus before the capsule is discharged. The drug is given in capsules of sizes No. 0, 1, 2, and 3.

Fitzgerald (*6*) has described and figured a balling gun and speculum suitable for the administration of capsules of carbon tetrachlorid to foxes, and has discussed the technic of this treatment. The balling gun has a rigid barrel, prolonged by a flexible tip, and is provided with a flexible plunger.

The survival of 2 animals out of 3 after such a large dose as 18.6 cc. by drench is especially interesting in view of the report by Allen (*1*) on the effect of various anthelmintics on foxes. He found that chloroform was extremely toxic for foxes, killing 50 per cent of the animals treated, even though the dose used was too low to secure satisfactory anthelmintic action. He found that thymol in a dose which was 87.8 per cent effective against hookworms killed 18.7 per cent of the animals treated; when the dose was reduced to give an efficacy of 33 per cent, the fatalities totaled 5.8 per cent of the animals treated. It would therefore appear that carbon tetrachlorid solves the question of safety in treating foxes for hookworms, for the therapeutic dose rate of 0.3 cc. per kilo for removing hookworms (the dose for dogs and the one which has been used on foxes with satisfactory results in a number of cases which have come to our attention) has a safety factor of 9 when compared with the dose rate of 2.7 cc. per kilo which was survived by 2 out of 3 foxes to which it was administered in a drench. Allen (*2*) has since stated that he has used

carbon tetrachlorid in comparatively few cases with apparently good results. Subsequently he (*3*) has reported that in the treatment of 399 foxes 95 per cent of the hookworms present were removed. Seven foxes were killed by the treatment, probably from inhalation intoxication in most cases, due to accidents in administration. Allen (*4*) has reported critical tests on 23 foxes and finds an efficacy of 93 per cent against hookworms. A small quantity of castor oil was given by him in connection with the administration of the carbon tetrachlorid in capsules.

Jeffreys (*24*) has reported the treatment of 69 fox pups, 17 days old to 5 weeks old, without evidence of discomfort or ill effects, which indicates the safety of carbon tetrachlorid since drugs in general—and this is true of anthelmintics—are much more toxic to young animals than to mature animals. Jeffreys found the drug 100 per cent effective against hookworm and ascarids. He also reports that it is 100 per cent effective in removing intestinal flukes from foxes. According to Jeffreys, the use of carbon tetrachlorid "is worth thousands of dollars to every fox rancher."

In another paper, Jeffreys (*23*) states: "An efficient remedy for the removal of hookworms was unknown a year ago, and the blue fox rancher was greatly handicapped. Now . . . we have an efficient remedy in the form of carbon tetrachloride. With this problem of parasites practically solved the rancher will have greater success in developing the blue fox industry."

When capsules break in the mouth and the drug enters the lungs, animals can be revived in many cases by artificial respiration. In view of the fact that carbon tetrachlorid and its vapors are very heavy, it would probably be advisable to hold the animal with its head down to assist in mechanically clearing the lungs.

At the present time effective treatment for the removal of hookworms from foxes is a matter of special interest, since fur foxes can not be imported into the United States if they are infested with hookworms. Previously, the majority of foxes could not be admitted to this country, since they are usually infested and no drug then known appeared to be safe and effective. Allen (*4*) also found carbon tetrachlorid effective in removing ascarids from foxes. We have no reports at present as to its value in removing whipworms, but what is true of it in the case of the same worms in dogs will probably be true of it in the case of the worms in foxes.

TESTS ON SWINE

The worm of greatest importance in the case of swine in this country is the large roundworm, or ascarid, (*Ascaris lumbricoides*). It has added interest because it has been generally accepted by parasitologists of late years that the ascarid of swine and the ascarid of man are identical; this conclusion is reached by Bakker (*5*) in a recent detailed study. Inasmuch as the tests on dogs indicated that carbon tetrachlorid would remove ascarids from these animals, and in view of the fact that chenopodium, the most effective drug known for the removal of ascarids, is of variable composition and occasionally gives rise to toxic effects, especially when given with inadequate purgation, we carried out some tests of carbon tetrachlorid against ascarids in swine as follows:

A series of 4 pigs, weighing 18 to 24 pounds, were given 6 cc. of carbon tetrachlorid in 30 cc. of castor oil by drench and were killed 5 days later with the following results: Pig 3381 passed 17 ascarids and had

1 dead, partly digested ascarid in the stomach and 7 live ascarids in the small intestine post mortem; pig 3382 passed 34 ascarids and had none post mortem; pig 3383 passed 9 ascarids and had 10 live ones post mortem; pig 3384 passed 2 ascarids and had 4 post mortem. The treatments were, respectively, 75, 100, 47, and 33 per cent effective. There were 63 ascarids removed (including the worm killed but not yet passed) and 21 left, an efficacy for the series of 75 per cent. The dose rate here is from 0.55 to 0.7 cc. per kilo, the efficacy rising from 33 per cent to 100 per cent with the increasing dose rate. These animals were apparently not properly fasted.

A second series of 5 pigs was treated as follows: Pig 3385, weighing 23 pounds, was given 12 cc. of the drug in 12 cc. of castor oil and died within 48 hours without passing any feces; post mortem it had 5 whipworms and 3 nodular worms; the digestive tract showed areas of high-grade inflammation. Pig 3386 was held as a control animal without treatment; post mortem it showed 17 ascarids, 14 whipworms, and 1 lungworm. Pig 3387, weighing 20 pounds, was given 6 cc. of carbon tetrachlorid in hard capsules; it passed 2 ascarids; post mortem it had 13 whipworms; this animal had an abscess involving the upper esophagus and posterior larynx, probably due to the administration of the capsules by the balling gun. Pig 3388, weighing 21.5 pounds, was given 6 cc. of carbon tetrachlorid in one large capsule, the animal dying a few minutes later, apparently as the result of the entrance of the capsule into the trachea.

A summary of the findings in this series shows that in the case of 2 animals weighing 9 to 10 kilos, each of which was given 6 cc. of carbon tetrachlorid in capsules in one case and in 25 cc. of castor oil in the other, all the ascarids were removed. The animals had been fasted from noon of the previous day, and under these conditions the dose rate of 0.6 to 0.67 cc. per kilo was entirely effective in removing ascarids. The use of a balling gun to administer capsules produced an abscess in the case of one of these animals, and in the case of another animal, where a large capsule was used, it caused death by forcing the capsule into the trachea. A dose of 12 cc. of carbon tetrachlorid in an equal quantity of castor oil, or about double the dose rate of 0.6 cc. per kilo, caused the death of one of the animals treated. If this dose had been administered in a larger quantity of castor oil so as to diminish the irritation and lessen the amount of inhalation of carbon tetrachlorid, it might have been tolerated. The results of this series of tests suggest that the drug may be satisfactorily administered to swine, from the standpoint of safety, in castor oil after an adequate fast (24 hours at least, and possibly 36 hours would be better), and that when so administered it will in many cases remove all ascarids present when given at the rate of 0.6 cc. per kilo.

A series of tests on a large animal were made as follows: Pig 3384, weighing 125 pounds (about 57 kilos), was given 8 cc. of carbon tetrachlorid in 60 cc. of castor oil; no worms were passed during the next 4 days; it was then given 16 cc. of carbon tetrachlorid in 60 cc. of castor oil; no worms were passed during the next 5 days; it was then given 32 cc. of carbon tetrachlorid in 60 cc. of castor oil; the next day the animal passed 1 ascarid, no other worms passing during the next 4 days; it was then given 40 cc. of carbon tetrachlorid in 60 cc. of castor oil; no worms were passed during the next 5 days; it was then given 32 cc.

of carbon tetrachlorid in 60 cc. of castor oil; no worms were passed during the next 4 days; post mortem no worms were found. The one worm passed after a treatment following a fast of 24 hours. The effective dose rate was 0.56 cc. per kilo. This animal showed no bad clinical effects or post-mortem lesions from the administration of repeated doses totaling 128 cc., or about 4 ounces, of carbon tetrachlorid in the course of 3 weeks.

Tests on another large animal were made as follows: Pig 3350, weighing 140 pounds (about 64 kilos) was given 32 cc. of carbon tetrachlorid in 60 cc. of castor oil; no worms were passed during the next 6 days; it was then given 40 cc. of carbon tetrachlorid in 90 cc. of castor oil; 1 ascarid was passed during the next 7 days; it was then given 50 cc. of carbon tetrachlorid in 120 cc. of castor oil; no worms passed during the next 4 days; post mortem there were no ascarids, but many nodular worms. The effective dose in this case was at the rate of about 0.63 cc. per kilo. The animal showed no clinical symptoms or post-mortem lesions as a result of the administration of 120 cc. (about 4 ounces) of carbon tetrachlorid in 13 days.

A slightly larger dose rate was tested as follows: Pig 3375 was given 40 cc. of carbon tetrachlorid in 100 cc. of castor oil; it passed 1 ascarid during the next 7 days; post mortem it had no ascarids, but had a large number of nodular worms. This animal weighed about 100 pounds (45 kilos), so the dose rate was about 0.89 cc. per kilo.

Having established by the foregoing series of tests that the effective dose rate necessary for the removal of all ascarids present in swine is at least 0.6 cc. per kilo for animals of various weights when properly fasted, we returned to the treatment of a series of small pigs as follows: Pigs 1a, 2a, and 4a, weighing 41, 37, and 34 pounds, respectively, were given 15 cc. of carbon tetrachlorid in 60 cc. of castor oil; and pigs 5a and 6a, weighing 35 and 26 pounds, respectively, were given 12 cc. of carbon tetrachlorid in 60 cc. of castor oil. During the next 6 days pig 1a passed 4 ascarids, pig 5a passed 26 ascarids, and pig 6a passed 1 ascarid, the other pigs passing no worms. All the pigs were then given 15 cc. of carbon tetrachlorid in 60 cc. of castor oil, and during the next week pig 2a passed 1 ascarid. All the pigs were then given 30 cc. of carbon tetrachlorid in 60 cc. of castor oil, no worms being passed during the next 5 days. Fecal examinations of these pigs had shown a decreasing number of worm eggs, but since the largest number were from the pen of pig 4a, which had passed no worms, this animal was killed and examined post mortem. No worms were found, so the eggs present were evidently due to contamination of the pen floor from old feces. The initial doses for 3 of the pigs were at the rate of 0.77 to 1 cc. per kilo, and for the other 2 pigs at the rate of 0.8 to 1 cc. per kilo. This dose rate apparently removed all the ascarids from all animals except one, and in the case of this animal a second dose at the same rate as the first, 0.8 cc. per kilo, removed 1 ascarid, probably the only worm present, since the third dose at an increased rate of from about 1 to 1.66 cc. per kilo removed no worms from any of the animals.

It appears from the foregoing that the effective dose rate of carbon tetrachlorid for ascarids in swine is at least 0.6 cc. per kilo of weight, the best method of administration being in 1 to 4 ounces of castor oil, according to the weight of the animal. Preliminary fasting for 24 to 36 hours appears to be essential. Occasionally, doses at a higher rate will fail to

remove the ascarids present, but the drug removed all ascarids present from 9 out of 12 swine examined post mortem. Animals will tolerate doses of 1.66 cc. per kilo of weight and will tolerate repeated doses equivalent to a dose rate of 2.24 cc. per kilo in 3 weeks (5 doses) or 1.87 cc. per kilo in 13 days (3 doses). Reports which have come to our attention indicate that the dose rate of 1 ounce for animals weighing 100 pounds should not be exceeded in the case of small pigs. At the rate given in the foregoing tests, the treatment appears to be quite safe, but the bulk of drug required for the removal of all ascarids present may prove to be an objection. The effective dose of chenopodium for removing ascarids from swine is about 4 cc. for a 100-pound animal. The dose of carbon tetrachlorid for an animal of this size would be about 27 cc., or approximately an ounce of carbon tetrachlorid, as compared to a dram of chenopodium. It would therefore appear that chenopodium must be regarded as a more potent drug for the removal of ascarids from swine. Carbon tetrachlorid appears to be of little value for removing whipworms and nodular worms from swine. Of 140 ascarids collected from the manure, 62 per cent were passed the first day after treatment, 9 per cent the second day, 25 per cent the third day, and 4 per cent the fourth day.

We are informed by Doctor Peters that carbon tetrachlorid is proving effective in removing the thorny-headed worms of swine, but owing to a lack of animals infested with these worms we have no experimental evidence in regard to this.

To determine the lesions produced by a lethal dose of carbon tetrachlorid in swine, the following experiments were carried out:

Pig No. 1G, weighing 41 kilos (90 pounds), was given 100 cc. of carbon tetrachlorid, a dose rate of 2.44 cc. per kilo, by stomach tube, a horse stomach tube being used. The pig appeared dull and stupid soon after treatment. The next day it appeared to feel better, but it died the following night, within 48 hours after treatment. On post-mortem examination no carbon tetrachlorid was found in the digestive tract, but a quantity was found in the thoracic cavity. In connection with this finding, a tear was found in the thoracic portion of the esophagus, and it seems probable that the large stomach tube used had perforated the esophagus, allowing the drug to enter the thoracic cavity. The liver showed a striking bronzing of the lobules and on microscopic examination by Dr. Leigh Giltner, of the Pathological Division of the Bureau of Animal Industry, a degeneration and capillary hemorrhage were found. The pathological conditions present in this and the following case will be discussed by Doctor Giltner in a separate paper.

Pig 2G, weighing 34 kilos (75 pounds), was given 100 cc. of carbon tetrachlorid, a dose rate of approximately 3 cc. per kilo, by stomach tube, a horse catheter being used as a stomach tube. The animal appeared somewhat stupid and died during the night, in 12 to 18 hours after treatment. On post-mortem examination a considerable quantity of carbon tetrachlorid was found in the small intestine and stomach, together with a considerable quantity of fluid. The liver showed the bronzed appearance as in the case of the previous pig and also some evidence of hemorrhage. The microscopic appearance was similar.

It appears from the findings in the case of pig 2G that a dose of 3 cc. per kilo is rapidly fatal and gives rise to distinct lesions of the liver. The previous experiments showed that pigs would tolerate carbon tetrachlorid at a dose rate of 1.66 cc. per kilo. The minimum lethal dose is therefore

between 1.66 and 3 cc. per kilo. Since the therapeutic dose rate is 0.6 cc. per kilo, the margin of safety is between 2.66 and 5, a very small margin for swine as compared with the large margin in the case of carnivores, poultry, and some other animals. Swine are therefore rather poor subjects for treatment with carbon tetrachlorid or other drugs acting on the liver—a fact probably associated, among other things, with the prevalence of parasitic hepatic cirrhosis in swine. That carbon tetrachlorid might prove unsatisfactory for swine on actual test was pointed out in an article by Hall (*14*).

TEST ON HORSES

In a paper by Hall (*15*) tests of carbon tetrachlorid for removing bots and worms from horses have been reported. Two animals were given carbon tetrachlorid alone in doses of 25 and 50 cc. The smaller dose removed 23 per cent of the *Gastrophilus intestinalis* from the stomach and 29 per cent of the *G. veterinus* (*G. nasalis*) from the duodenum, or 24 per cent of all bots present. The larger dose removed 23 per cent of the bots from the stomach and 100 per cent of the bots in the duodenum, or 25 per cent of all bots present. Since the smaller dose is equivalent to the therapeutic dose of carbon bisulphid (CS_2) which will remove all the bots present, carbon tetrachlorid is evidently inferior to carbon bisulphid as a remedy for bots, although the efficacy shown by carbon tetrachlorid is superior to that of anything that has been used, except carbon bisulphid, no other drug having shown even the 24 to 25 per cent efficacy shown by carbon tetrachlorid.

A number of stomach worms (*Habronema* sp.) were found post mortem in the stomach of one horse, none having been found in the manure. In our opinion, this can be taken only to prove that carbon tetrachlorid and other potent drugs will not kill these worms when they are buried in the mucosa. It appears entirely probable that carbon tetrachlorid, carbon bisulphid, or chenopodium will kill those individuals with which they come in contact. It further appears that as a rule the appearance of the dead worms in the manure is not to be expected. A dead worm in the stomach is in effect a very small amount of protein material in an environment where protein digestion is being carried on, and it is to be expected that the worm will be wholly or in large part digested before it can leave the stomach unless it is killed near the pylorus and is very promptly carried out by peristalsis. Such fragments of Habronema as might escape in this manner would almost certainly be completely digested and disintegrated in their passage through the small and large intestines. The journey through the large intestine of the horse may require days and even weeks for individual objects, and Hall, Smead, and Wolf (*20*) found dead bots in the large intestine as late as 17 days after the administration of carbon bisulphid. Confirmatory evidence in regard to the digestion of dead worms in the stomach will be given later in connection with our studies on treatment for stomach worms in sheep. It therefore appears that the technic used here is not adapted to obtaining accurate information in regard to the efficacy of anthelmintics on worms in the stomach and that the findings in regard to treatments for these worms must be reviewed with reference to the occurrence of gastric digestion of the worms.

The carbon tetrachlorid in the smaller dose removed the few ascarids present, suggesting that this drug is perhaps as effective as carbon bisulphid against these worms.

In the doses used, carbon tetrachlorid killed all the Strongylus present in the two horses. This efficacy is in line with the 100 per cent efficacy this drug displays against other bloodsucking worms, such as hookworms in the dog and others we shall mention in this paper. That this drug is able to kill these worms in the large intestine of the horse is apparently due in part to the low solubility and relatively slight volatility of the drug which enable it to reach the large intestine in quantities sufficient to kill the worms, and this should also be correlated with the fact that this drug does not depress the unstriated musculature of the digestive tract and stop peristalsis.

While the carbon tetrachlorid removed large numbers of cylicostomes, it also left large numbers. It appears to be inferior to chenopodium for the removal of these worms.

In the paper in question (*15*), additional tests on horses, with mixtures of carbon tetrachlorid and carbon bisulphid were reported. The tests indicate that the mixture is not a very useful one. We may state briefly that for removing the bots and worms from the stomach and small intestine, carbon bisulphid is the drug of choice, removing all bots and ascarids in almost all cases when given in doses of 6 drams in hard capsules to animals properly fasted. In our opinion it will probably kill specimens of Habronema not protected by the mucosa or in some other manner. For the removal of worms from the large intestine, chenopodium is the drug of choice, removing practically all Strongylus, cylicostomes and related strongyles, and the pinworms, when given in doses of about 4 to 5 drams to animals properly fasted. These two drugs supplement each other in eliminating the bots and worms from the digestive tract of the horse. Carbon tetrachlorid appears to be quite effective in removing ascarids and Strongylus, but to eliminate practically all the gastrointestinal parasites noted above it would need to be supplemented by carbon bisulphid to remove the bots and chenopodium to remove the cylicostomes and pinworms, whereas carbon bisulphid and chenopodium alone are sufficient. It does not therefore appear that carbon tetrachlorid can be used for general purposes in removing worms from horses, though it might be the drug of choice in some cases for the specific purpose of removing ascarids or Strongylus or both. It is the best single drug of the three, apparently, since it removes the ascarids and Strongylus and removes some bots and cylicostomes.

We have as yet too little information to permit of definite statements as to the toxicity of carbon tetrachlorid for horses. The horse given 50 cc. weighed about 900 pounds (409 kilos), the drug being given at a dose rate of slightly over 0.12 cc. per kilo. This horse, an old and weak animal, died on the fifth day after treatment and showed a number of chronic pathological conditions which could not be attributed to the drug. Since carbon bisulphid in 3 doses of 3 fluid drams each, a total of 9 fluid drams or about 35 cc., will occasionally kill old or weak animals, the only warranted assumption is that carbon tetrachlorid is probably safer than carbon bisulphid, the lethal dose rate per kilo being relatively low for both drugs and low for carbon tetrachlorid as compared with the lethal dose rate in the case of such animals as carnivores and poultry.

TESTS ON SHEEP

The results of our tests of carbon tetrachlorid on sheep have been very satisfactory from the standpoint of efficacy. In one series, 4 lambs, which were found on fecal examination to have a moderate infestation with stomach worms, were given, respectively, 12, 18, 24, and 48 cc. of carbon tetrachlorid, the drug being mixed in each case with 2 ounces of castor oil and administered by means of a dose syringe. We recovered 9 stomach worms (*Haemonchus contortus*) from the manure of one, 8 from another, and 2 from a third. On post-mortem examination all the animals were found to be entirely free from stomach worms. This 100 per cent efficacy is superior to the results obtained from the use of copper-sulphate solution by Hall and Foster (*19*) and is additional evidence showing the particular efficacy of this drug against the bloodsucking worms. As the fecal examinations indicated that all of these animals were moderately infested with stomach worms, the failure to obtain these worms from the manure of one animal and the collection of such small numbers in the case of the other three are evidently due to the digestion of the worms in the stomach, as in the case of Habronema in the horse. The stomach worms lie among the laminae of the abomasum and this, doubtless, further insures digestion by holding them until they are digested, except in the case of the worms near the pylorus. That this is true is evident from the findings of Hall and Foster. Following the administration of a 1 per cent solution of copper sulphate to 3 sheep, they recovered, respectively, 120, 240, and 314 stomach worms from the manure. Two other sheep from the same flock were given copper sulphate in capsules; each passed 41 stomach worms, and had, respectively, over 4,000 and over 6,000 of these worms post mortem. It seems quite reasonable to assume that there was probably an average of 5,000 worms originally present in all 5 animals and that less than 10 per cent of the worms killed by the copper-sulphate solution were collected from the manure.

A second experiment carried on by us indicates much the same results. Of 3 lambs, all heavily infested as shown by fecal examination, 1 was held as a control and the other 2 were given, respectively, 15 and 30 cc. of carbon tetrachlorid in 2 ounces of castor oil. Three stomach worms were collected from the manure of one animal and no worms of this sort were present post mortem in either animal. The control animal had 612 stomach worms post mortem. We must conclude that the carbon tetrachlorid was 100 per cent effective in removing the stomach worms from 6 sheep and that the failure to obtain worms from the manure in two of these cases is due to the digestion of the worms in the stomach, which also accounts for the small number recovered in the other cases. It may be further said that stomach worms collected from the manure after treatment are usually represented only by small fragments, frequently the portion found showing the characteristic vulva region in the female. Entire worms are rarely found. It may also be mentioned that apparently carbon tetrachlorid itself has a destructive action on hookworms and may have a similar action on other worms.

A third series of tests on sheep was conducted as follows:

Sheep No. 3316.—Weight 80 pounds (about 36 kilos); given 12 cc. in one No. 10 and one No. 12 capsule; passed no worms in 4 days; post mortem, 36 small trichostrongyles and 6 *Moniezia* sp.

Sheep No. 4c.—Weight 80 pounds (about 36 kilos); given 8 cc. in one No. 11 capsule; passed 59 hookworms in 2 days and none the next 2 days; post mortem, 2 *Œsophagostomum columbianum*, 1 *Moniezia* sp., and a few *Gongylonema scutatum*.

Sheep No. 5c.—Weight 68 pounds (about 31 kilos); given 4 cc. in one No. 10 capsule; passed 40 hookworms in 1 day and none the next 3 days; post mortem, 14 *Haemonchus contortus*, 1 *Œsophagostomum columbianum*, 95 small trichostrongyles, and a few *Gongylonema scutatum*.

Preliminary fecal examinations indicated that all these sheep were infested with stomach worms, and it is possible that the worms present in 2 of the sheep were destroyed by the carbon tetrachlorid and digested, leaving no evidence in the form of recognizable fragments in the manure. However, it appears from this experiment that a dose of 4 cc. of carbon tetrachlorid is too small to remove all stomach worms present and that it is necessary to use at least 8 cc., and possibly more, to remove all these worms.

In our first series of 4 sheep no hookworms were present. In the second series the 2 animals treated with 15 and 30 cc. in castor oil passed 5 and 2 hookworms, respectively, and had none post mortem. The control animal had 18 hookworms. In the third series the 2 infested animals treated with 4 and 8 cc. in capsules passed 40 and 59 hookworms, respectively, and had none post mortem. In view of the efficacy displayed by this drug against other hookworms and against blood-sucking strongyles in general, it seems probable that the same complete efficacy would have been shown if large numbers of hookworms had been present. This is very promising, especially in view of the removal of all hookworms present by a dose as small as 4 cc. In the experiments reported by Hall and Foster (*19*) the copper-sulphate treatment failed to remove any hookworms, and the same was true of chloroform. Gasoline removed only 5 per cent of these worms. Chenopodium removed 66 per cent and petroleum benzine 73 per cent. The chenopodium, however, had an indicated efficacy of only 4 per cent against stomach worms and the petroleum benzine an indicated efficacy of 88 per cent in comparison with the ascertained 100 per cent efficacy of carbon tetrachlorid against stomach worms in doses of 12 cc., and perhaps 8 cc., and against hookworms in doses as small as 4 cc.

In the first series of 4 sheep the carbon tetrachlorid in doses of 12 to 48 cc. removed 30 per cent of the nodular worms and 30 per cent of the whipworms. In the second series of 2 sheep the carbon tetrachlorid in doses of 15 and 30 cc. removed 90 per cent of the 189 whipworms present from one animal and all of the 12 whipworms present from the other. This looks promising. Whipworms, as we have already noted, are very difficult to remove with one treatment, but if sheep will tolerate large doses of carbon tetrachlorid, this may furnish us with a satisfactory treatment for the removal of these worms. In the second series of 2 sheep the treatment removed only 3 per cent of the 192 nodular worms present in the case of the sheep from which it removed over 90 per cent of the whipworms. The drug removed all of the 12 nodular worms present in the other animal. In the third series the drug failed to remove any of the 3 nodular worms present when given in doses of 4 and 8 cc.

In our first series of 4 sheep we found that the carbon tetrachlorid removed 82 per cent of the 801 small trichostrongyles belonging to the

genera *Nematodirus*, *Cooperia*, *Ostertagia*, and *Trichostrongylus*. This is a surprisingly good result, since these small worms have been resistant to the drugs heretofore tested on sheep. In the case of these worms and other small worms, the principle has been laid down and accepted by parasitologists that small worms are more difficult to remove than large ones. Certainly it has required more time and investigation to find any treatment whatever for their removal. That carbon tetrachlorid will fail to remove a large percentage of these worms at times was shown in our second series of 2 sheep, in which the drug removed 27 per cent of the trichostrongyles present in one animal and removed only about 3 per cent of 1,100 trichostrongyles present in the other animal. In this case the worms left were mostly Cooperia, Ostertagia, and Trichostrongylus, the drug being apparently most effective against the Nematodirus present. This is of interest, since Nematodirus is apparently a bloodsucker and is apparently a more injurious parasite in the case of sheep than any of the other trichostrongyles named. In the third series doses of 4 and 12 cc. apparently failed to remove any of the 121 trichostrongyles present.

Carbon tetrachlorid was of no value in removing tapeworms from sheep, removing only 1 and leaving large numbers. One sheep had 1,149 specimens of *Moniezia* post mortem. This agrees with the findings in the case of dog and chicken tapeworms.

The two lambs used in our second experiment were selected as the weakest and most unthrifty of a lot of lambs. The one which was given 30 cc. of carbon tetrachlorid weighed only about 33 pounds (15 kilos) and was down and apparently dying the day we killed these two lambs. Whether the carbon tetrachlorid contributed anything to the bad condition of the animal on this day is problematical. However, it is commonly true that very sick or weak animals are intolerant of anthelmintic treatment, and it is possible that the drug may have injured this one. Such animals are practically worthless and if they die after treatment, little or nothing is lost. Since most of the sheep used in the tests were off feed for a time following treatment, it appears that the safety factor of this drug for sheep is not very large. In this connection it may be recalled that Hall and Foster (*19*) found sheep intolerant of chloroform, one animal dying after a dose of 5 cc. The nature of the ruminant stomach probably favors rapid absorption of volatile drugs.

TESTS ON CATTLE

The following tests on cattle were conducted:

Heifer No. 1009.—Weight, approximately 250 pounds (approximately 114 kilos); dose, 100 cc. of carbon tetrachlorid in 350 cc. of castor oil, after fasting 36 hours. Passed 399 stomach worms (*Haemonchus contortus*) during first 2 days, 396 hookworms (*Bunostomum phlebotomum*) during first 6 days, 234 nodular worms (*Proteracrum radiatum*) during first 6 days, and 4 whipworms (*Trichuris ovis*) during first 3 days; post mortem, on sixth day, 426 hookworms and 1 tapeworm (*Moniezia* sp.).

Heifer No. 1002.—Weight, approximately 175 pounds (approximately 80 kilos); dose, 100 cc. of carbon tetrachlorid in hard capsules, after fasting 36 hours. Passed 8 stomach worms and 8 nodular worms in first 2 days, and 453 hookworms in first 4 days; post mortem, on fourth day, 4 hookworms in the small intestine and 2 hookworms in the cecum.

Heifer No. 1009, a clinical case of hookworm disease, was off feed for the period following treatment, but the animal was killed too soon to determine whether it would have returned to good condition and shown any benefit from the treatment. Heifer No. 1002, also a clinical case of hookworm disease, went down following the treatment and was found dead the morning of the post-mortem examination. Evidently the treatment was not very well tolerated by these animals. Post mortem, heifer No. 1002 showed the following: Some ecchymotic areas in the small intestine and cecum and a pronounced gelatinous infiltration about the stomach and large intestine; kidneys and liver apparently normal; urinary bladder slightly congested and containing a quantity of mucus; hemorrhagic areas on the heart, with some evidence of degeneration of the heart muscle. While some of these lesions may have been associated with the use of carbon tetrachlorid, it is also true that they may have been associated with the hookworm infestation. These animals were from a herd that had lost a number of animals from hookworm disease and some of the lesions are those associated with an anemic condition. The gelatinous infiltration about the stomach and large intestine is a condition more readily associated with long-standing anemia and edema than with a toxic action extending over a period of four days. However, the safety of the drug in the doses given is open to question under the circumstances until further investigations have been carried out. From the findings in the case of sheep, it seems reasonable to conclude that ruminants will not tolerate carbon tetrachlorid to the same extent that carnivores will and that the safety factor is much smaller. The doses used in the case of these two heifers were at a rate of approximately 0.88 cc. and 1.25 cc. per kilo, a very low rate by comparison with that tolerated by dogs.

Dr. W. A. Barnette writes in a letter of October 3, 1922:

> It seems to me from what few clinic cases I have tried carbon tetrachloride on that I am getting excellent results, giving to yearlings one-half ounce in a half pint of olive oil, and to cows weighing from 700 to 800 pounds 1 ounce in 1 pint of olive oil.

Dr. W. K. Lewis writes in a letter of October 16, 1922:

> A few days since we treated 19 head of cattle, consisting of 9 adults and 10 calves and yearlings, for stomach worms, using carbon tetrachlorid in capsules. The dosage for the adults averaged about 22 cc. Within 36 hours 4 head of adults were dead, all the adult animals becoming sick, while none of the yearlings and calves were affected The dosage for the yearlings and calves was relatively higher than for the adults, one heifer having been given 10.5 cc. These animals were kept off feed for 24 hours before treatment and were allowed to graze about one-half to three-fourths hour after treatment. They were taken off feed again in about 1 hour to be milked and again turned on feed. One or two of the animals showed some bloating, but the others showed only a listless appearance until they would get down and appeared to be in pain, dying within a few hours. There was no bowel action from any of the cows that died and the survivors were very slow to respond to salts.
>
> Post-mortem on one of the animals showed considerable food in the rumen, which had a sour fermented odor; there was no evidence of inflammation in the intestinal organs, only the entire mesenterium contained a large amount of coagulated jelly-like blood serum. From the lesions we thought that we got a local anesthesia of the intestinal tract and the animals died from autointoxication.

It is difficult to account for the results obtained in Doctor Lewis's cases. As a rule, anthelmintics and most other drugs are more toxic for young animals than for mature animals, and this appears to be true for carbon tetrachlorid. The doses used by Doctor Barnette were larger than those used by Doctor Lewis and were followed by good results clinically. The doses used by us were distinctly larger and the smaller

animal was found dead the morning of the fourth day after treatment, the larger animal being killed by us the sixth day to conclude the experiment. The results contrast very strikingly with the deaths in 36 hours of mature animals given much smaller doses. No plausible explanation occurs to us in this connection, but it is possible that further work along this line will throw some light on the subject.

As regards efficacy, it will be seen that the drug as given in our cases was 100 per cent effective against stomach worms in the case of both animals; 48 per cent effective against hookworms in the case of the first animal and 99 per cent in the case of the second animal, regarding the two hookworms found in the cecum as removed by the drug; 100 per cent effective against nodular worms in the case of both animals; 100 per cent effective against whipworms in the case of the first animal, the only one infested; and 0 per cent effective against tapeworms in the case of the first animal, the only one infested. The drug was therefore extremely effective in removing stomach worms, nodular worms, and whipworms; it removed from almost half to almost all the hookworms present, but was of no value in removing tapeworms. These results warrant further investigations to determine the actual utility of this drug in treating cattle infested with stomach worms, nodular worms, hookworms, and whipworms to determine whether it can be given in a dose which will be effective without injury to the animal treated.

TESTS ON MONKEYS

In the experiments on monkeys, carried on in cooperation with Dr. G. C. Lake, of the Public Health Service, we gave doses of twice to five times the indicated human dose of 3 cc., the latter based on the fact that the dose for the adult person and the average-sized dog is usually the same for anthelmintics and many other drugs. These monkeys weighed from 4.5 to 7.5 pounds. All monkeys survived the dose given, one of them weighing 4.5 pounds receiving 12 cc., or approximately 6 cc. per kilo. Taking the weight of an average-sized man as 70 kilos, and assuming that man could tolerate this drug at the same rate, the equivalent dose for the man would be about 420 cc. At this rate the indicated therapeutic dose of 3 cc. would have a safety factor of 140. While it is not assumed that this is true, nevertheless it appears that this drug should prove quite safe for man. Its complete efficacy against the blood-sucking worms, hookworms in dogs, Strongylus in horses, and stomach worms in sheep indicates that it will probably prove equally effective against hookworms in man.

The monkeys in this experiment were kept under observation for one month and then were released from this experiment. An attempt was made to determine something of the anthelmintic value of the drug in the case of monkeys, but owing to a misunderstanding the attendant did not collect the feces of the first 24 hours after treatment. The feces for the next 48 hours contained 1 whipworm and 34 heterakids, apparently *Subulura distans*. The drug is evidently of some value in removing heterakids from monkeys, but owing to the fact that no post-mortem examination was made and the doses used were very high, little can be concluded as to its value.

In a recent paper, Lake (*25*) has published the results of some further tests on monkeys. He finds that monkeys weighing 2.21 to 2.63 kilos tolerate 12 to 16 doses of 1, 2, 3, and 5 cc. each at intervals of 2 to 3 days

for a period of 30 to 41 days and a total of 16 to 66 cc. without any evidence of bad effects, and that the two animals receiving the highest totals, 48 and 60 cc., showed no macroscopic or microscopic lesions post mortem as a result of the treatment. These doses are 10 to 40 times greater for weight of animals treated than the indicated dose of 3 cc. for man. He concludes that the toxicity of carbon tetrachlorid is very low for monkeys and probably for man also, and that repeated doses would probably be well tolerated by man.

TESTS ON MAN

One of the writers (*16*) has reported a test in which he took 3 cc. of carbon tetrachlorid without any precautions as to food and on the same day did a day's work, including a post-mortem examination of some horses. He smoked more than usual and took more than the usual amount of exercise. No unpleasant effects were experienced and the only sensation noticed was a feeling of warmth in the stomach during the first half hour. The other writer (Shillinger) has since made the same test with much the same result, except that the drug could be tasted at intervals for several hours and there was a slight transient dizziness. Both of these effects were perhaps associated with a slight condition of indigestion already present when the drug was taken. Dr. Karl Hanson, of the Biological Survey, has also taken a similar dose without bad effects. We are informed by Dr. E. Encisco that the drug was tried on human patients in Bogota, Colombia, for whipworms about four years ago and then abandoned, since it was not satisfactory for removing these worms. As we have noted, whipworms are difficult to remove, owing to their location. They constitute, therefore, a decidedly unsatisfactory test object for anthelmintic efficacy in general. Doctor Encisco did not recall the dose used. At the present time this drug is being tested in human medicine for the removal of hookworms on the basis of the findings in the case of the dog hookworm. If it proves equally effective and safe, its obvious advantages may make it the drug of choice for removing these worms from man.

An editorial in the Lancet, London, for February 25, 1922,[4] reports that the administration of a single dose of carbon tetrachlorid to natives of the island of Fiji resulted in the removal of 98 per cent of the hookworms present. In Nature for May 27, 1922,[5] there is a statement as follows: "A telegram has been received from Fiji reporting the successful treatment of more than 12,000 hookworm cases by carbon tetrachloride with 90 per cent of cures with one dose, and the removal of 98 per cent of the worms."

Leach (*26*) has a detailed report of the treatment with carbon tetrachlorid of 14 persons, 13 of whom were infested with hookworms, in Ceylon. Previously, some of these patients had been given two treatments with betanaphthol, totaling 110 grains (7 gm.), with the removal of an average of 8 hookworms per patient. Carbon tetrachlorid removed an average of 58.7 hookworms per patient, this drug being given in doses of 3 cc. to 3 patients, 4 cc. to 8 patients, 5 cc. to 1 patient, and 10 cc. to 1 patient. The patient receiving 10 cc. was given 2 cc. more 15 days later. This man was a prisoner condemned to be hanged. He passed 55 hookworms, 4 ascarids, and 67 pinworms. On post-mortem examination the digestive tract contained no hookworms or ascarids but had 3,492 pin-

[4] CARBON TETRACHLORIDE IN ANKYLOSTOMIASIS. *In* Lancet, v. 202, p. 391. 1922.
[5] TREATMENT OF HOOKWORM CASES BY TETRACHLORIDE. *In* Nature, v. 109, p. 688. 1922.

worms and 32 whipworms. The spleen, liver, and kidneys were apparently normal. All the patients were negative for hookworms on fecal examination 3 weeks after treatment. The drug was given without purgation and had a marked cathartic action, the bowels moving in most cases in 3 to 5 hours after treatment. Urine examinations were made in two cases before and after treatment and showed no alterations as a result of the treatment. The pulse and the diastolic and systolic blood pressure were noted before and after treatment and showed that the heart action was practically undisturbed. The patients experienced only slight giddiness and a sensation of weight in the stomach for the most part. One patient had diplopia and nausea. The patient receiving 10 cc. complained of giddiness, nausea, and drowsiness, but after sleeping 4 hours the nausea disappeared and the giddiness diminished.

Owing to a lack of capsules, the drug was given in 20 cc. of water, which might account, in our opinion, for some symptoms as a result of inhalation. The ineffectiveness of the drug against pinworms and whipworms might also be correlated to some extent with the fact that the patients were allowed food immediately after treatment. In our opinion this might lower the efficacy of the drug.

Nicholls and Hampton (*28*) have given a further report on the tests of the drug in Ceylon. They report its administration to 20 students, 18 to 25 years old, in an agricultural school, 3 cc. being given without subsequent purgation. They state that the students carried out the regular program of the day without restrictions and that—

> Not one of them was inconvenienced by the drug, and they all continued their day's work in the gardens or at the school house.

Microscopic examination of the feces 10 days later showed hookworm eggs in the feces of only 2 students, and the group passed an average of 36 hookworms each, together with a total of 13 ascarids. The drug was then given to 64 students from 7 to 17 years old, in quantities of 1 to 3 cc., food being allowed an hour later. These writers say:

> One 12-year-old boy vomited when he had finished his meal, but the manager informed us that this child often vomited. . . . The drug acted as a mild aperient. The patients passed an average of 43.3 hookworms and a total of 260 ascarids, 15 whipworms, and 4,945 pinworms. . . . The discharge of Trichuris and Oxyuris indicates that the drug acts throughout the intestinal tract, for we have not previously seen Trichuris expelled after the use of other anthelmintic drugs, or *Oxyuris vermicularis* expelled in such large numbers.

Ten days after treatment fecal examinations of 54 of these students showed hookworm eggs in 6 cases.

The drug was given to four children 3 to 6 years old, previously treated once or oftener with chenopodium, removing 9, 35, 52, and 52 hookworms. It was given to five children 4 to 9 years old, of whom one had malaria, one an enteric disorder, and three a pyrexia of unknown origin continuous in two cases and irregular in one case. These children passed from 6 to 167 hookworms each and were in no way inconvenienced by the treatment.

These writers state:

> The drug may be administered safely in doses of 10 to 20 minims to children of 3 and 4 years of age, even when they are seriously ill from various cases. It aids the expulsion of *Ascaris lumbricoides* if it is followed by a purgative, but it is not as effective as chenopodium in killing this worm. The drug does not seriously deteriorate on keeping. Many children were given doses of carbon tetrachloride which had been stored in the laboratory for 3 years. It is more valuable than chenopodium for campaigns against hookworm disease because (a) patients do not object to its taste; (b) it is not necessary to precede or follow the administration of it by a purge; (c) it is much

more efficient than chenopodium and has not the depressing effects of that drug; (d) it is much cheaper than any drug that has been used; (e) it can be prepared in a high degree of purity, and a chemically pure preparation should always be used; (f) the person who is being treated can do his usual day's work. Children when 1 year of age may be given 10 minims of carbon tetrachloride with safety, and this dose should be increased by 2 minims for each year of apparent age. Thus a child of 10 would receive 28 minims, a youth of 16, 40 minims (2.5 cc.), and an adult dose should be from 50 to 80 minims (3 to 5 cc.), according to the size of the patient.

These authors report a second case of a prisoner treated with carbon tetrachlorid and then executed. This man was given 6 cc. on one occasion and a similar dose 13 days later. A week later the man was executed and found to have no ascarids or hookworms on post-mortem examination; they state that 4 ascarids were passed after the first treatment. The organs showed no degeneration on microscopic examination. Hampton (*22*) refers to these same prisoners and notes that in the second case 1 whipworm and 8 pinworms were found post mortem. With reference to the 20 students referred to by Nicholls and Hampton (*28*), he states that 13 reported a slight headache and giddiness, 4 claimed that they felt burning or rather tingling sensations in the body, and 4 had no symptoms whatever. Hampton states:

All admitted that the symptoms they felt were too slight to mention. Two of those treated had previously taken chenopodium and 1 had taken thymol; all 3 stated that they preferred to take carbon tetrachloride. The head master stated that in his opinion the students were not inconvenienced at all by taking the treatment. He had seen 2 previous classes take chenopodium and found that they suffered considerable inconvenience at the time.

As regards cost, he says that chenopodium costs 30 shillings a pound and is given in a dose of 1.5 cc., followed by magnesium sulphate; carbon tetrachlorid costs 3 shillings a pound and is given in a dose of 3 cc. without a purgative. As regards efficacy, he states that "One 1.5 cc. dose of oil of chenopodium gives microscopical cures in from 30 to 50 per cent of the patients treated, while one 3 cc. dose of carbon tetrachloride gives microscopical cures in 90 per cent of the cases treated."

McVail (*27*), according to a review, has given children, aged 12, 1 dram (3.75 cc.) of carbon tetrachlorid on 2 successive days without ill effects. A dose of 1 dram to a very old man was followed by irregularity of the pulse and slurring speech. The largest dose he gave an adult was 70 minims (about 4.35 cc.) on 2 successive days. He reports that the drug is a soporific, and that at a leper asylum 51 patients were given a dose of 1 fluid dram each one evening, and states—

and all slept so soundly that a burglar was able to remove the contents of the rice godown during the night. . . . It does not appear to aggravate albuminaria and may be given with confidence in cases of kala-azar complicated with ancylostomiasis during the remissions of temperature, though kala-azar cases stand chenopodium badly. Carbon tetrachlorid is of little value against Ascaris, Trichuris, and *Hymenolepis nana*. On the other hand, this drug appears to be almost specific for threadworms [pinworms]. In 13 cases Oxyuris worms were found in the stools after a single treatment with carbon tetrachlorid, and in 4 cases after double treatment, though Oxyuris ova had been found during the previous microscopic examination only in 3 cases out of the 17. [It is exceptional to find eggs in the feces in human infestations with pinworms, as the gravid female migrates to the rectum and passes out with the eggs stored in the uterus.]

The above report in regard to the efficacy of carbon tetrachlorid in removing pinworms from man should be correlated with the findings of Nicholls and Hampton (*28*), who report the passage of 4,945 pinworms by a group of 64 students, an average of 77 worms each, assuming that all were infested, and with the findings of Leach (*26*), who reports the

passage by a prisoner of 67 pinworms and the presence post mortem of 3,492 pinworms. The findings in Leach's case, where a relatively large dose, 10 cc. followed later by 2 cc., had been given, suggest that carbon tetrachlorid does not have a dependable high efficacy against the human pinworm, although it will remove some of the worms present in many cases.

One of the writers (Hall) has taken carbon tetrachlorid twice since the test reported by him (*16*). On that occasion the drug was taken in hard capsules. In a second test it was taken in a soft gelatine capsule to determine the apparent time in which such capsules open in the stomach. The capsule contained 1.5 cc. of carbon tetrachlorid and was from a lot that was probably 6 months old at the time this was taken. The capsule was taken 2 hours after breakfast, and 20 minutes later a slight sensation of dizziness and of discomfort at the stomach was noticed, the sensation passing away in the course of a minute. No further sensations were noticed, although no precautions were taken in regard to food, smoking, or exercise. This suggests that soft capsules will open in the stomach in the course of a half hour. However, the tests of this drug and of choloroform, as administered in soft capsules to dogs, point to a certain loss of efficacy against hookworms where soft capsules are used.

Since the reports on the administration of the drug show that it is being given in water, with which this drug does not mix, a third test was made in which 3 cc. of the drug was taken in 30 cc. of milk. Carbon tetrachlorid being a fat solvent mixes somewhat better with milk than with water, but the drug is nevertheless left at the bottom of the milk. The mixture was not unpleasant to take, but left a somewhat unpleasant taste in the mouth. In a half hour a sensation of light-headedness and drowsiness came on and this persisted to some extent for the next 5 hours. The effects were more noticeable than in the previous tests, and the odor of the drug could be detected in eructations for several hours. At the end of 5½ hours vomition occurred, and after drinking some coffee this occurred a second time 6½ hours after taking the drug. The food eaten an hour after treatment was digested, but some carbon tetrachlorid evidently remained in the stomach, causing nausea by slow absorption. Since fats and oils, especially such bland oils as olive oil, tend to remain for long periods in the stomach, as Asnis has noted for olive oil, it might be assumed that fats and oils tend to hold carbon tetrachlorid in the stomach and that such substances are contraindicated in connection with the use of this drug. The experiments on cats to ascertain the effect of fats and oils on the absorption of carbon tetrachlorid, as already reported in this paper, did not confirm the idea that these substances increased the toxicity of carbon tetrachlorid. However, this point should be kept in mind by those using the drug, as a matter on which information is needed. It might be of interest to note that the experimenter was the subject of a Mayo short-loop posterior gastrojejunostomy from 11 to 12 years previous to the time these tests were made.

One of the writers (Shillinger) on another occasion took 2 hard gelatine capsules each containing 1.5 cc. of carbon tetrachlorid directly after the midday meal. There was the same sensation of dizziness that was felt when the drug was taken by him several weeks previously, one-half hour before eating at midday, and the drowsiness, as experienced by Hall, was noticed. There was no particularly disagreeable sensation of irritation or discomfort but rather a desire to rest or sleep, which persisted in mild form for about 3 hours.

SUMMARY

Carbon tetrachlorid has a pronounced selective action on bloodsucking worms, as shown by its high efficacy against hookworms in dogs, foxes, cats, sheep, and cattle, *Strongylus* in the horse, stomach worms in sheep and cattle, and *Nematodirus* in sheep.

It has a high efficacy against ascarids in dogs, cats, foxes, swine, and horses, being less effective, apparently, than chenopodium against ascarids in swine and more effective against ascarids in horses. It is less effective than chenopodium against ascarids in man, but its approximate efficacy for the doses used can not be stated at present.

It is not effective in removing pinworms from horses, but evidently has some efficacy against the related heterakids of monkeys and of birds. It will remove some pinworms from man in many cases, but its approximate efficacy can not yet be stated.

It is inferior to chenopodium for removing cylicostomes from horses, but is much superior to any drug yet tested for removing small trichostrongyles from sheep. It will remove some nodular worms from sheep but it exhibits a variable efficacy in this connection, removing none in 2 cases, 3 per cent in 1 case, 64 per cent in 1 case, and 100 per cent in 1 case. In the large doses used on cattle it removed all the nodular worms present.

In view of the results obtained and the apparent tolerance for large doses of this drug which some species of animals show, it may prove possible to remove whipworms from some species of animals with this drug by giving doses so large that their bulk will in most cases insure the entrance of this drug into the cecum. One hundred per cent efficacy was obtained in some cases in removing whipworms from dogs, sheep, and cattle.

Carbon tetrachlorid is inferior to carbon bisulphid for the removal of bots, but is superior to any drug other than this which has been tested against bots.

Carbon tetrachlorid is not of value in removing tapeworms from chickens, dogs, sheep, cattle, or man, and will probably be of little value against tapeworms in any animals. We have seen a report in an Italian newspaper to the effect that this drug is specifically valuable in removing *Tænia solium*, but the report is evidently based on the reviewer's belief that the hookworm is the armed tapeworm.

Its value in removing spirurids and Capillaria from chickens is problematical and requires further investigation.

Carbon tetrachlorid has not been tested on such forms as the common liver fluke (*Fasciola hepatica*). Floris (7) has reported carbon bisulphid as of value against these flukes. His work has not been followed up by other workers, however, and more information is needed in regard to this. If carbon bisulphid is effective, carbon tetrachlorid might also prove effective. Jeffreys (*24*), as already noted, states that carbon tetrachlorid is 100 per cent effective in removing intestinal flukes from foxes. He does not report his experiments and we have had no opportunity to make tests along this line.

Many patients report no symptoms following the oral administration of carbon tetrachlorid in therapeutic dose. Of the symptoms reported the most common appear to be a transient dizziness, slight headache, and a sensation of weight, warmth, or slight discomfort at the stomach.

Other symptoms which are sometimes manifested are tingling or burning sensations and drowsiness. Rarely, usually following the administration of rather high doses, the drug may cause nausea, vomition, irregularity of pulse, and slurring speech.

CONCLUSIONS

Carbon tetrachlorid is an effective drug in removing certain worms. It is especially valuable for removing bloodsucking strongyles. It has a rather high efficacy against ascarids in certain species of host animals. It may prove of value in removing whipworms from some kinds of animals if experience bears out the existing findings to the effect that large doses of the drug may be safely tolerated, making it possible to insure the entry of the drug into the cecum. It has some efficacy against heterakids in the large and small intestines.

Enough evidence has accumulated in the published literature and in our experiments to warrant the statement that the efficacy of a drug against worms in the stomach can not be accurately ascertained by the technic employed by us, owing to the digestion of the dead worms in the stomach. It is evident that carbon tetrachlorid and some other drugs are highly effective against certain species of worms occurring in the lumen of the stomach, more effective than the evidence in worms passed indicates.

Carbon tetrachlorid when taken by mouth, and the inhalation of the drug avoided, appears to be an uncommonly safe drug, for most of the species of animals tested. It appears that the danger from inhalation is greater for carnivores than for man. It has in most instances a large safety factor for the therapeutic dose necessary to remove approximately 100 per cent of a number of important species of worm parasites. This drug has the advantage of being a very simple compound, which can be easily tested chemically to determine its purity, and a chemically pure product can be purchased at practically any drug store in civilized countries.

Carbon tetrachlorid has the additional advantage of being cheap. It is much cheaper than chenopodium or thymol, though not so cheap as the copper-sulphate solution for removing stomach worms from sheep. Its 100 per cent efficacy would appear to warrant further investigations as to its use against stomach worms of sheep, since the worms left by the copper-sulphate solution serve to maintain the infestation on pastures. Its value in this connection should also be weighed against that of the tobacco-copper-sulphate solution which Guberlet (*8*) finds superior to the copper-sulphate solution without tobacco.

On a comparison of doses of carbon tetrachlorid based on weight of animal, the tolerance of the drug by the various species of animals used is about as follows: Chickens will tolerate 20 cc. per kilo, the minimum lethal dose not being ascertained, from which we may surmise that birds will prove very tolerant of this drug. Turkeys tolerate doses of 1 cc. per kilo, and we may assume from the results with chickens that they will tolerate much higher doses. Dogs will tolerate 16 cc. per kilo, the minimum lethal dose not being ascertained. Cats will tolerate 8 cc. per kilo, but some animals are killed by approximately 9 cc. per kilo. Foxes tolerate 2.7 cc. per kilo administered in a drench, and we may surmise that carnivores in general will prove very tolerant of this drug. Rabbits will tolerate 5 cc. per kilo, the minimum lethal dose apparently being close to 10 cc. per kilo, from which it appears that

rabbits, and probably the closely related group of rodents, will tolerate relatively large doses, but are not so tolerant as birds and some carnivores. Monkeys will tolerate 6 cc. per kilo, the minimum lethal dose not being ascertained, and are therefore probably about as tolerant of the drug as rabbits. Adult swine will tolerate 1.66 cc. per kilo, but the tolerance is less for young animals, and swine are evidently less tolerant of carbon tetrachlorid than are the animals already referred to. The fact that swine are not more tolerant may be correlated with the common occurrence of hepatic cirrhosis in swine and the effect of the drug on the liver. Sheep will tolerate 1.3 cc. per kilo, but the minimum lethal dose may be about 2 cc. per kilo. Young cattle will tolerate 0.88 cc. per kilo, with an indicated minimum lethal dose of about 1.25 cc. per kilo. From the foregoing we may conclude that ruminants are less tolerant of this drug than are birds, carnivores, rabbits, monkeys, or swine, this decreased tolerance apparently agreeing with the ascertained diminished tolerance of ruminants for such volatile drugs as chloroform and probably being associated with the presence of the four stomach divisions of ruminants with a resultant rapid absorption of volatile drugs. The tolerance of approximately normal horses for carbon tetrachlorid is yet to be ascertained, since the animal which died 5 days after a dose of 0.12 cc. per kilo was an old, weak animal with pronounced lesions of a chronic nature.

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STRUCTURE, PHYSICAL CHARACTERISTICS, AND COMPOSITION OF THE PERICARP AND INTEGUMENT OF JOHNSON GRASS SEED IN RELATION TO ITS PHYSIOLOGY [1]

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INTRODUCTION

These investigations were undertaken in hope of explaining some features of the behavior of Johnson grass seeds during their initial dormancy, their period of after-ripening, and their germination. As has been shown elsewhere (*15*),[2] Johnson grass seeds belong to that group whose well-matured embryos are never appreciably dormant, the dormancy of the intact fruit being imposed by its outer, nonliving structures. These include (1) the closely adhering, hard, brittle scales, (2) the fused pericarp and inner integument. Removal of the former hastens the germination and increases the germination capacity of the caryopses, whether these are freshly gathered, are fully after-ripened, or are in process of after-ripening, but does not appreciably affect the rate of after-ripening. Furthermore, removal of the fused pericarp and integument by corrosion with concentrated sulphuric acid or even its removal over one side of the embryo by means of a sharp needle induces the complete germination within three or four days even of freshly gathered grains which, without such treatment, would scarcely germinate at all in weeks or months.

It has also been shown (*15, 16*) that the germination of Johnson grass seeds is highly dependent upon the maintenance of alternating temperatures, that this sensitiveness to temperature conditions disappears upon the removal of the seed coverings, and that certain chemical substances exert a stimulating action upon their germination, particularly after the removal of the caryopses from the inclosing scales. These facts suggested that a study of the physical and chemical characteristics of the seeds, and especially of their pericarps and integuments, might help to explain their physiological behavior.

In marked contrast to the dormancy and germination physiology of Johnson grass seeds, Sudan grass seeds germinate very readily at ordinary temperatures, constant or alternating, without the lapse of any considerable period of after-ripening, and without any special preliminary treatment. Sudan grass seed has therefore been used for comparison with Johnson grass seed in the studies herein reported. The greater part of the work was done in the Hull Botanical Laboratory of the University of Chicago, and the remainder in the Seed-Testing Laboratories of the United States Department of Agriculture in Washington.

Both Johnson grass and Sudan grass have been described under several different names, and they have frequently been assigned to different species. Mr. A. S. Hitchcock considers Johnson grass as *Holcus halepensis*

[1] Accepted for publication July 2, 1921.
[2] Reference is made by number (italic) to "Literature cited," p. 221–222.

L. (*Sorghum halepensis* Pois) and Sudan grass as subspecies of the same, which he calls *Holcus halepensis sudanensis* (Piper) Hitchcock (*Andropogon halepensis sudanensis* Piper). The very close taxonomic relationship of the two forms increases the interest which attaches to the marked physiological differences between their seeds.

STRUCTURE OF THE FRUITS AND CARYOPSES OF JOHNSON GRASS

I. EXTERNAL APPEARANCE

Oakley (*25*), Vinall (*30*), and Youngblood and Conner (*32*) discussed the external appearance of the fruits of Johnson grass in comparison with those of Sudan grass. Hillman (*17*) later described in detail the fruits of these two kinds of grass, both with the scales on and with the scales removed.

Figure 1, adapted from figure 4 in Hillman's paper, shows the external characters of the fruit of Johnson grass. The unhulled fruit or spikelet (A) is about 0.2 inch long and a little less than one-half as wide as long. It bears at its proximal end a smooth regular scar (a). The caryopsis (B) with its hyaline lemma is inclosed in two, straw-colored to black overlapping scales, the glumes. The outer scale, or lower glume, is convex and spear-shaped (A, I).

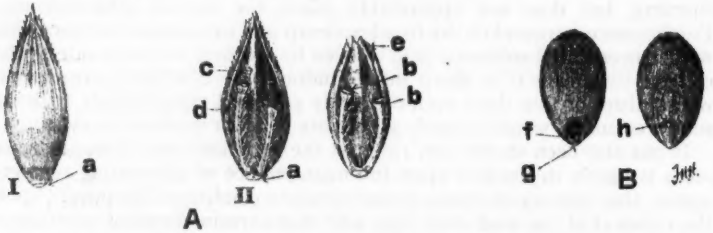

FIG. 1.—Johnson grass spikelets and caryopses: A, Unhulled spikelets, showing convex outer surface at I and flat inner surface at II; B, caryopses, showing scar of the spikelet (a), appendages of the spikelet (b, b), upper glume (c), overturned edge of the lower glume (d), broken ends of the glumes (e), depressed area where the pericarp extends over the hilum (f), scar of the caryopsis (g), and embryo (h)

The inner scale, or upper glume (c), is nearly flat, but slightly keeled. Its outer edge is inclosed by the overturned edge of the outer scale (d). The flattened inner side of the unhulled fruit usually bears two appendages, a rachis segment and the pedicel of a sterile spikelet (b, b). The distal ends of the glumes frequently are jaggedly broken (e) in many of the fruits of commercial lots. We have found that sufficient rubbing to cause a large amount of such breakage or even the very careful cutting away of the distal half of the glumes increases the germination of dormant lots even if none of the caryopses are removed from the scales.

The caryopsis (fig. 1, B) is about three-fourths as long as the unhulled fruit, oval or oval-elliptical in shape, dark reddish brown in color. The side of the caryopsis which was next to the outer scale is nearly flat and bears at its proximal end a small, roundish, very dark, and somewhat depressed area (f) where the pericarp extends across the hilum. At the extreme proximal end is the scar of the caryopsis (g). The side which was next to the inner scale is somewhat convex. At its proximal end is located the fairly large embryo (h), the position of which is clearly marked by a somewhat lighter color than that of the rest of the caryopses.

II. INTERNAL STRUCTURE OF THE CARYOPSIS

A large number of botanists have investigated the embryos of various grasses. Several of these, among whom are Bruns (8), Pammel (26), Guerin (14), Kennedy (19), and Sargant and Arber (27), have described the embryos and other organs of the caryopses of species of Andropogon, Sorghum, and other species of the tribe Andropogoneae.

Figure 2 shows a median sagittal section of a Johnson grass caryopsis. No attempt is made here to distinguish between integument and pericarp, as these structures are discussed and illustrated in later sections.

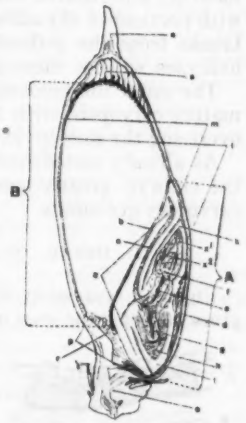

FIG. 2.—Median sagittal section of a Johnson Grass caryopsis: A, embryo; B, endosperm, showing scutellum (a), its epithelium (b), its central procambium strand (c), portion of wing of scutellum rolled up over the axial organs of the embryo (d), mesocotyl (e), epicotyl with its embryonic leaves X^1 and X^2 (f), radicle (g), root cap (h), root sheath or coleorhiza (i), epicotyl sheath or coleoptile (k), aleurone layer (l), fused pericarp and seed coat (m), remnant of style (n), pedicel with remnants of lodicules (o), region of the hilum and micropyle (p), compressed and empty endosperm cells (r), and elongated distal cells of the integument (s). X 25.

Besides the axial organs, the rather large embryo (A) includes the scutellum (a), the root sheath, or coleorhiza (i), and the epicotyl sheath, or coleoptile (k). The radicle (g) is directed toward the proximal end of the caryopsis. Its tip is covered by a well-developed root cap (h). The epicotyl (f), with the first two convolute leaves (x^1 and x^2) well formed and the rudiments of later leaves, extends toward the distal end of the caryopsis. Between the radicle and the epicotyl is a short, internode-like structure, the mesocotyl (e), which is variously interpreted as the fused hypocotyl and stalk of the cotyledon (27) or the elongated primary node (31).

The scutellum is expanded laterally into two wings which fold up around and almost wholly envelop the axial organs of the embryo. A section of the upper edge of one of these wings is shown at (d). Wherever the scutellum is in contact with the endosperm (B) the cells of its lower cell layer are elongated into the form of a columnar epithelium (b), which secretes diastase and possibly other enzyms for the conversion of the stored food of the endosperm. Along the entire length of that portion of the scutellum underlying the mesocotyl and epicotyl extends a central procambium strand (c), which enters the axial organs at the point of their insertion upon the scutellum and which sends off about a dozen small lateral branches and a short branch which extends under the radicle. These branches ramify throughout that face of the scutellum which is in contact with the starchy endosperm. Upon the germination of the caryopsis the elements of the central strand become differentiated, with the formation of spiral tracheae, and the cells of the columnar epithelium greatly elongate. These morphological changes can also be induced by wounding the endosperm region of the caryopsis or by a diseased condition of embryo or endosperm. The elongation of the epithelial cells, either generally over the whole face of the scutellum or locally, is always accompanied by corrosion of the starch grains in the underlying endosperm cells.

Just inside the coverings of the caryopsis (m) and extending entirely around the embryo and endosperm except at the region of the hilum and

micropyle (p) is the aleurone layer (1), which comprises a single layer of cells.

Between the hilum, the starch-bearing endosperm, and the proximal end of the scutellum lies a light-colored mass of large, irregular, compressed, empty endosperm cells (r).

The coverings of the caryopsis consist of the fused product of the pericarp and inner integument—the latter of a single layer of cells which, at the distal end of the caryopsis, are much elongated and have very thick inner walls. The figure shows these elongated cells of the inner integument (s), a portion of one of the persistent styles (n), and the pedicel (o), with portions of the adhering lodicules. Usually, however, the caryopsis breaks from the pedicel at a point just distal to the insertion of the lodicules, so that these are not present on the hulled caryopsis.

The outer integument and nucellus have entirely disappeared in the mature caryopsis, with the possible exception of a portion of the former overlying the micropyle.

As already stated, complete or partial removal of the coverings over the embryo greatly increases the readiness with which Johnson grass caryopses germinate.

III. DETAIL OF THE PERICARP AND INNER INTEGUMENT

Figure 3 represents the pericarp and inner integument of a Johnson grass caryopsis as seen in cross section about midway from the proximal end to the distal end of the caryopsis. The drawings in this figure were made with camera-lucida and oil immersion lens from paraffin sections 15 π in thickness, stained with iron alum haematoxlyin. The material used had not been bleached or otherwise altered before embedding in the paraffin. Figure 3, A, was drawn from the flat (endosperm) side of the caryopsis opposite the end of the scutellum, and figure 3, B, from the rounded side over the end of the embryo (1) and adjacent endosperm cells (2).

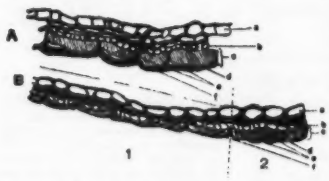

FIG. 3.—Cross sections of the fused pericarp and inner integument of Johnson grass caryopsis: A, from flat (endosperm) side of caryopsis at a point opposite the end of the scutellum; B, from rounded (embryo) side over the end of the embryo (1) and adjacent portion of the endosperm (2), showing outer epidermis of the pericarp (a), inner epidermis of the pericarp (b), inner integument (c), its inner walls (d), its side walls (e), and its outer walls and solidified contents of the cells (f) × 325.

The pericarp consists of several layers of cells of which only the outer epidermis (a) and the inner epidermis (b) are clearly distinguishable. The intervening layers consist of greatly compressed thin-walled cells, in which narrow cell lumena and intercellular spaces appear only at irregular intervals. One of these layers is the chlorophyll-bearing layer of the earlier stages of development of the caryopsis and in the mature fruit frequently contains starch grains. The outer epidermis is continuous and slightly undulating in surface contour and consists of rather large rectangular cells, with relatively thin walls and large lumena. The inner epidermis, as in other Gramineae, is fragmented longitudinally into long tubular cells, extending lengthwise of the caryopsis and connected with each other by their end walls, occasionally by their lateral walls, and by cells extending diagonally at infrequent intervals. In cross section these cells usually appear circular or broadly elliptical as in figure 3.

The inner integument (c) is a single-cell layer, continuous except at the hilum. The cells are much larger than any of the cells of the pericarp.

Their inner walls (d) and side walls (e) are very thick and dense, and dark brown in color; their outer walls are much thinner and less dense. There is a tendency for the outer walls to collapse into the cell lumen, which itself frequently becomes nearly or quite filled with a solidified, granular, slightly brown or yellowish mass of substance.

The outer walls and solidified cell contents are usually difficult to distinguish from each other. Together they are represented by the cross-lined areas (f).

Toward the distal end of the caryopsis the cells of the inner integument gradually increase in size and their inner walls increase in thickness, culminating in the great development shown in figure 2 (s). Over the embryo the inner integument is much thinner and somewhat lighter-colored than over the greater part of the endosperm—a feature which largely accounts for the lighter color of the embryo region of the caryopsis as contrasted with the endosperm region. The integument decreases in thickness also over the flat, or endosperm, side of the caryopsis proximally from the position indicated in figure 3, though on this side of the caryopsis its inner walls are always thick, dense, and very dark-colored. Proximally the inner integument ends in areas of special development at the micropyle and the hilum which will be described in detail in the following pages.

Figures 4 to 8, showing specialized areas of the pericarp and inner integument of Johnson grass caryopses, were all drawn with camera lucida from freezing microtome sections of fully imbibed seeds, the sections having first been decolorized on the slide with Javelle water, stained with methylene blue, and mounted in 75 per cent glycerin solution. The decolorizing process entirely removed the solid contents of the integument cells and somewhat increased the size of all cells. As the aleurone layer usually remained attached to the inner integument in the sections even when nearly all of the endosperm fell out, this layer is shown in the figures representing areas where it is present.

PERICARP AND INNER INTEGUMENT AT THE DISTAL END OF THE CARYOPSIS

Figure 4 represents a median sagittal section through the coverings of a Johnson grass caryopsis at its distal end. The inner integument (a) is much thicker than the combined thickness of the pericarp and the aleurone layer, and is much thicker toward the flat (endosperm) side of the caryopsis (A) than toward the embryo side (B), though this relatively great difference does not persist far from the distal end. The inner walls of the integument cells (1) are extremely thick and bear at intervals peculiar knoblike ingrowths (2) into the cell cavity. These ingrowths may be smooth but more frequently are studded with minute points. In the latter case, very infrequently one is found which before bleaching with Javelle water, but never after bleaching, exhibits double refraction in polarized light as if crystalline in structure. These ingrowths occur only near the distal end of the caryopsis. The long cells of the integument at the extreme distal end of the caryopsis sometimes extend far into the style and are broken off with the style by rough handling of the caryopsis. The relatively thin end walls of the integument cells at a little distance from the distal end are sometimes slightly folded (3) as if from the inward pressure of the pericarp, as it dries during maturation of the caryopsis.

The aleurone layer (b) is of relatively thick-walled cells, but the walls are not pigmented. Its thick walls, continuity, and persistent adherence to the integument are of interest in connection with the suggested protective roll of this layer in grass caryopses (7).

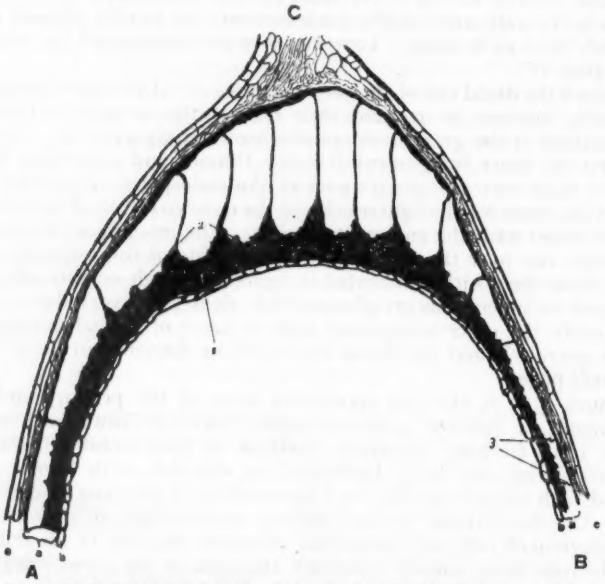

FIG. 4.—Median sagittal section through the coverings of a Johnson grass caryopsis at its distal end: A, flat, or endosperm side of caryopsis; B, embryo side of caryopsis; C, base of style, (a) inner integument, (1) inner walls of its cells, (2) inward thickenings of same, (3) end walls showing folds, (b) aleurone layer, (c) outer epidermis of pericarp. × 100.

Of the pericarp only its outer epidermis (c) is clearly and definitely distinguishable in freezing microtome sections. The figure shows the base of one of the persistent styles (C).

MICROPYLE AND SURROUNDING STRUCTURES

Figure 5 represents a median sagittal section through the micropyle of a Johnson grass caryopsis. The position of the micropyle with reference to other structures can be seen by comparing this figure with figure 2. The aleurone layer (fig. 2, l; 5, a) on the embryo side of the caryopsis extends several cells beyond the proximal end of the scutellum (fig. 2, a; 5, b), but falls several integument cells short of reaching the micropyle.

The micropyle itself (fig. 5, c) is closed by the cells of the inner integument (d,d) which has so crowded in from all directions as to become turned back upon itself externally, the cells from opposite directions coming together but without the walls fusing. The inner cells of the double layer thus formed (e) are greatly elongated in a radial direction and have very heavy, densely pigmented inner walls. The cells of the outer layer (f) are considerably shorter and their outer (morphologically inner) walls are thick and heavily pigmented. On the side toward the hilum this reversed layer of inner integument cells extends within 2 or 3 cells of the edge of the hilum (g) and forms a conspicuous hump. In

the opposite direction it extends only about half a dozen cells and its surface forms a regular flat contour with that of the single layer of cells of which the inner integument consists farther from the micropyle.

The pericarp over the micropyle as elsewhere consists of the very distinct outer and inner epidermises and between these 3 or 4 cell thicknesses of thin-walled, elongated, irregularly arranged cells. The outer epidermis (h) is of thick-walled cells with large lumena, rectangular in section; the inner epidermis (i) of small, thick-walled, closely crowded, heavily pigmented tubular cells, roundish or elliptical in sagittal section, but changing between the micropyle and the hilum to compressed, rectangular cells with much thinner walls. At this point the inner epidermis of the pericarp is underlaid by another layer of small, thick-walled cells, also heavily pigmented (k), which extends uninterruptedly over the micropyle from the hilum, thinning out and gradually disappearing distally. The origin of this cell layer is not clear, but it may be a persistent portion of the outer integument in which the micropylar opening is entirely obliterated. Over the area where the reversed outer layer of the inner integument ends next to the hilum is a mass of small, irregular, closely packed pericarp cells (l) whose very thick, densely pigmented walls are fused with the walls of the integument cells upon which they impinge. This group of cells around the margin of the hilum is continuous with the cells of the "closing tissue" of the hilum to be described in later pages. Thin-walled pericarp cells (r) fill the hilum.

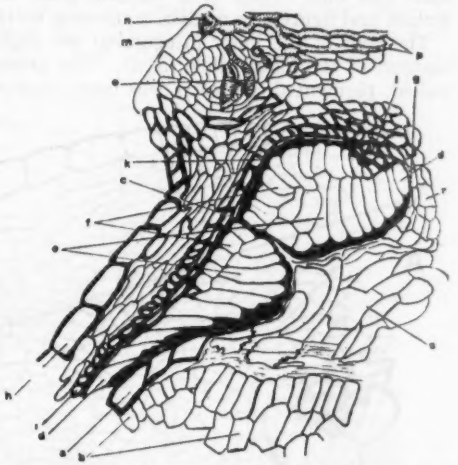

FIG. 5.—Mean sagittal section through the micropyle and neighboring structures of a Johnson grass caryopsis: a, aleurone layer; b, proximal end of scutellum; c, micropyle; d,d inner integument; e, its inner layer of cells at the micropyle; f, its outer layer of cells at the micropyle; g, edge of the hilum; h, outer epidermis of the pericarp; i, inner epidermis of the pericarp; k, layer of cells locally underlying the inner epidermis of the pericarp, possibly a persistent portion of the outer integument; l, group of closely packed, thick-walled pericarp cells whose walls seem to be fused with the walls of the inner integument cells; m, edge of irregular scar of the caryopsis; n, scalariform tracheids of the pedicel; o, coiled branch of tracheid system; p, tracheids in rows parallel to the surface of the hilum; r, pericarp cells which fill the hilum; s, large, empty, functionless endosperm cells which underly the micropyle. × 180.

Where the caryopsis breaks irregularly from the pedical (m) the pericarp is supplied with a group of scalariform tracheids (n), most of which end irregularly only a few cells from the break, while a few extend for a short distance in rows in a tangential direction over the hilum. A constant feature of this system of tracheids is its splitting into two branches, one of which ends abruptly in a coiled knot, a few elements of which are shown (o) in a direction toward the micropyle, while from the other branch extend the rows of tracheids over the hilum (p). These latter rarely extend over the circular hilum for more than one-third of its diameter, and the underlying tissue of the pericarp is entirely nonvascular.

Under the micropyle is a group of large empty functionless endosperm cells (s) which continue also under the hilum and gradually give place to the starchy reserve cells of the endosperm.

THE HILUM

Since in a caryopsis the seed never becomes detached from its pericarp there is, of course, no true hilum, or seed scar. There is, however, in the caryopsis of the Andropogoneae, a large opening through the inner integument in the position corresponding to the hilum. Figure 6 represents this hilar orifice of a Johnson grass caryopsis in median sagittal section and figure 7 in median transverse section.

The cells of the inner integument are slightly turned outward at the margins of the hilar orifice (a, a). The group of small, compact, thick-walled, pigmented, pericarp cells mentioned in the preceding section are

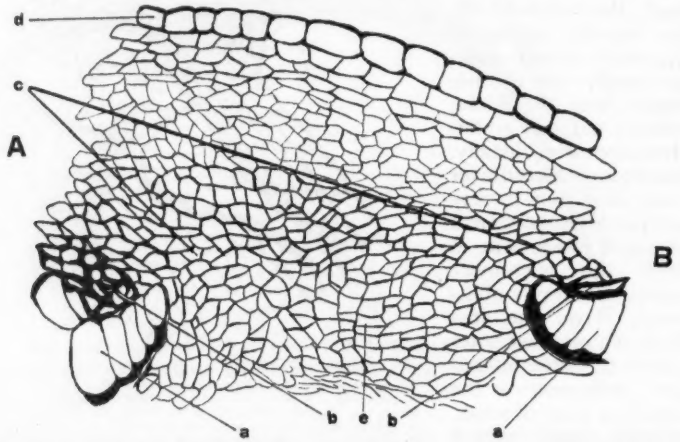

FIG. 6.—Median sagittal section through the hilar orifice of a Johnson grass caryopsis: A, side toward micropyle and endosperm; B, side toward embryo, showing inner integument (a, a), compact group of thick-walled pericarp cells (b, b), "closing tissue" of the hilar orifice (c), outer epidermis of the pericarp (d), pericarp cells completely filling the hilar orifice in the integument (e). × 275.

present outside of the margins of this orifice on all sides (b, b). They are particularly prominent in sagittal section on the side toward the micropyle (fig. 6, A) and are rather sparingly represented on the embryo side (fig. 6, B). Ninety degrees around the circumference of the hilar orifice from these points as shown in figure 7, they are very numerous, but not as thick-walled as at the longitudinal extremities of the hilar region. Stretching over this region from the points where these thick-walled pericarp cells fuse with the integument near the margins of the hilar orifice is a continuous stratum of several layers of pericarp cells with somewhat thickened walls (c), which, though forming a single tissue with the cells on both sides of them, differ from these in ways which are of great physiological significance. The radial contraction which characterizes all pericarp tissues in the dry, mature caryopsis, causing the very noticeable hilar depression, apparently reaches its maximum in this stratum, which also becomes intensely pigmented with a dark brown pigment and, with the thick-walled cells shown at b, b, forms a pro-

tective cover to the large hilar opening through the inner integument. This cover we have designated as the "closing tissue" of the hilar orifice. As will be shown later, it is largely impervious to solutes and highly resistant to the action of 50 per cent chromic acid. In its protective function it supplements the inner integument with which it is structurally united through the fusion of the respective cell walls. Its intense pigment, showing through the overlying layers of pericarp cells, forms a circular black area which is always conspicuous in an external examination of the entire caryopsis. Around this closing tissue in all directions the degree of pigmentation abruptly decreases, though there is no sharp line between the very dark central region and the surrounding cells. In fact, when completely bleached with Javelle water, the cells of this dark central region and those above and below it are almost identical in

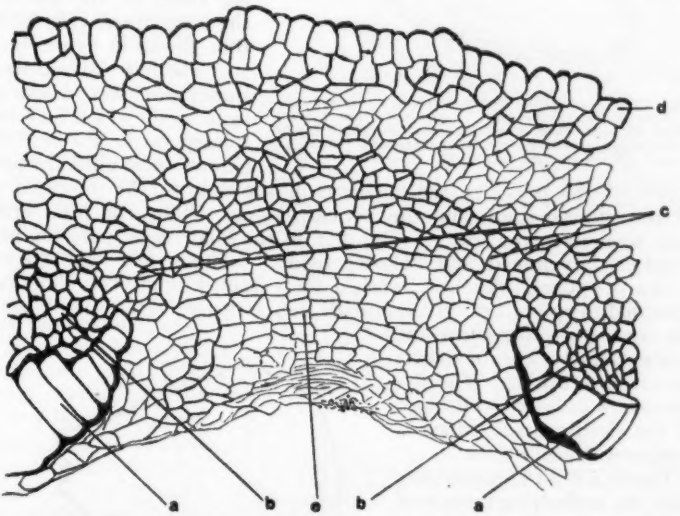

FIG. 7.—Median transverse section through the hilar orifice of a Johnson grass caryopsis: a, a, inner integument; b, b, compact group of thick-walled pericarp cells; c, "closing tissue" of the hilar orifice; d, outer epidermis of the pericarp; e, pericarp cells completely filling the hilar orifice in the integument. × 275.

appearance. The pericarp cells (e) within the margins of the hilar orifice are roughly cubical in shape and are arranged rather regularly in radial rows. In the absence of vascular bundle elements it is obvious that this mass of tissue—completely filling the hilar opening through the integument, and parenchymatous until pigmentation sets in during the maturation and drying of the caryopsis—acts as the only avenue for the conduction of nutrient materials from the vascular elements at the base of the pedicel and over the hilar region to the developing embryo and endosperm. Rows of long, thin-walled cells in the central layers of the pericarp tissue leading from the coiled branch of the vascular bundle of the pedicel and continuous with rows of cells within the hilar opening, doubtless also function as conducting elements. In the mature unbleached caryopsis, however, the dark central portion is strikingly differentiated from the surrounding structures. Moreover, the contrast here existing is accentuated by partial bleaching of microtome sections

with Javelle water. After the surrounding cells are almost completely decolorized, the compact, densely pigmented central portion, the "closing tissue," still remains very dark, with the individual cell walls wholly indistinguishable. Figure 8 represents in outline a median sagittal section of a Johnson grass caryopsis which had received this partial bleaching with Javelle water. On further treatment with Javelle water the cell walls in this dark closing tissue (f) also become completely decolorized and the compressed cells resume their cubical shape as shown in figures 6 and 7.

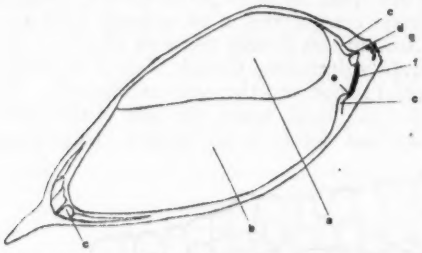

FIG. 8.—Median sagittal section through a Johnson grass caryopsis, partially bleached to show the "closing tissue" over the hilar orifice: a, embryo; b, endosperm; c, c, c, inner integument; d, micropyle; e, hilar margins; f, "closing tissue;" g, tracheids from the pedicel. × 27.

The relation of the closing tissue of the hilum to the integument cells is further shown in figures 9 and 10. The material from which these figures were drawn was from seeds which had been treated with 50 per cent chromic acid until the outer layers of the pericarp, the coverings of the embryo, the proximal end of the embryo itself, and the pericarp tissue within the hilar orifice had been dissolved away. This left in the hilar region only the more resistant integument and "closing tissue." These were removed with a sharp scalpel, bleached, washed and stained upon the slide, and mounted in 75 per cent glycerin with thin strips of tissue paper under the cover glass to protect the now extremely delicate structures from crushing. The figures show only one cell layer of the closing tissue, which is really several cells thick.

Figure 9 is an external view with the underlying margin of the hilar orifice shown as a continuous heavy line (a). The drawing was made with camera lucida and represents accurately the loose ends of the cell walls (b) at the margin of the tissue which had resisted the corrosive action of the chromic acid.

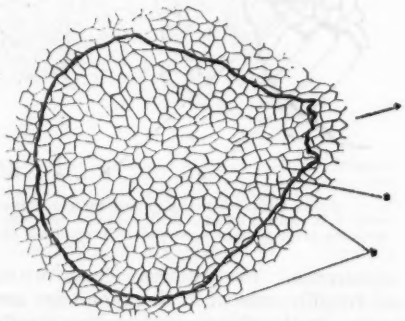

FIG. 9.—External view of the "closing tissue" over the hilar orifice of a Johnson grass caryopsis: a, underlying hilar margin; b, loose ends of cell walls of the "closing tissue," beyond which the tissue was destroyed by chromic acid; the arrow points in the direction of the micropyle. × 180.

Figure 10 is a view from the inner side of the hilum, looking out. The ends of the out-curving integument cells (a) present a stereoscopic appearance, while the much thinner-walled closing tissue (b) is shown in a lower focal plane as it appeared through the hilar orifice.

COMPARISON OF JOHNSON GRASS AND SUDAN GRASS FRUITS AND CARYOPSES

Sudan grass seed differs from Johnson grass seed in certain minor ways, some of which, however, are physiologically important. Both the unhulled fruits and the caryopses are slightly larger, flatter, and more

slender. The glumes are more fragile and more easily broken or removed, so that commercial lots of Sudan grass seed usually contain a considerably larger percentage of hulled caryopses than do Johnson grass seed. The caryopses are lighter colored, less glassy in appearance, and are more easily injured mechanically. The coat structures usually form conspicuous delicate folds or creases over the axial organs of the embryo, as the embryo shrinks during maturation and drying, while in Johnson grass caryopses the coverings are stretched rather tightly over the embryo even after the caryopsis is fully dried. In a Johnson grass caryopsis the micropylar prominence is usually the most proximal part of the inner integument, the embryo not extending farther forward than the micropyle. In a Sudan grass caryopsis, on the contrary, the end of the radicle and of the scutellum usually extend farther forward so that the inner integument is strongly arched forward from the micropyle to cover these organs. The Sudan grass embryo is therefore more exposed to mechanical injury than the Johnson grass embryo.

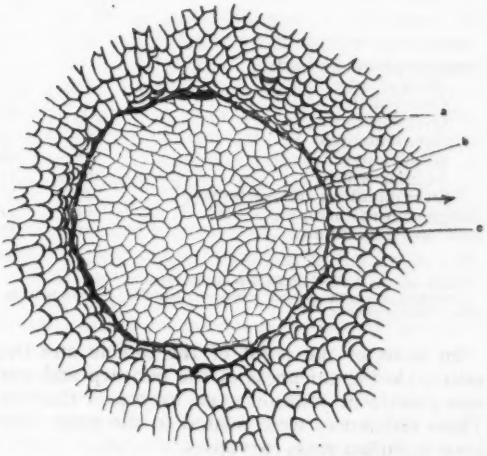

FIG. 10.—Internal view of the hilar orifice of a Johnson grass caryopsis with its "closing tissue:" a, ends of the integument cells; b, "closing tissue;" c, margin of the hilar orifice; the arrow points in the direction of the micropyle. × 180.

The micropylar structure is frequently less massive in Sudan grass caryopses than in Johnson grass caryopses. An open micropyle is rare in Johnson grass and somewhat more common in Sudan grass. One Sudan grass caryopsis was examined which had a nearly circular micropylar opening somewhat more than 100 microns in diameter through the integument.

Many Sudan grass caryopses are very light colored, and occasionally one is found which is almost white. In some such light colored caryopses the inner integument is poorly developed or almost lacking. In others it is as well developed as in the dark colored caryopses, but is less pigmented. Johnson grass produces no such light colored caryopses, and the inner integument is always well developed. Its thick inner wall, as well as the pericarp tissue, is more darkly pigmented than in Sudan grass caryopses.

MEASUREMENTS OF COAT STRUCTURES

Since removal of the coverings over the embryo of Johnson grass caryopses removes the restrictions to their germination at moderate and constant temperatures and makes them capable of germinating vigorously under the same conditions as Sudan grass caryopses, it was thought desirable to compare these coverings in the two kinds of seed. Measurements were therefore made of the minimum thickness of the coat

structures over various parts of the caryopses in 25-micron median sagittal freezing microtome sections of a large number of caryopses. Table I summarizes these measurements for several portions of the caryopses, each entry being the average of the minimum thickness for five or six caryopses.

TABLE I.—*Minimum thickness in microns, of coat structures of Johnson grass and Sudan grass caryopses* [1]

Area measured.	Small plump, dark Sudan grass caryopses.	Small well-matured Johnson grass caryopses.	Large, very light-colored Sudan grass caryopses.	Large, very dark Sudan grass caryopses.	Large, well-matured Johnson grass caryopses.
Integument and pericarp:					
Front of coleorhiza	34	29	32	28	34
Over radicle	31	22	28	23	24
Over coleoptile	29	21	30	26	21
Over scutellum	36	23	31	26	25
At micropyle [2]	78	66	79	52	96
At hilar orifice	80	71	65	64	83
Over middle of endosperm side	40	33	43	38	28
Integument at micropyle	35	42	52	48	58
Dark area over hilum	11	13	9	12	10

[1] Each entry is the average for several caryopses.
[2] The thickness at the micropyle was very variable, depending upon the manner in which the caryopses broke from the pedicel.

In front of the point of the radicle and over the hilar orifice and micropyle the thickness of the pericarp and inner integument combined was greater in Johnson grass caryopses than in Sudan grass caryopses. These differences were related to the more forward position of the embryo in Sudan grass caryopses.

Over all other portions of embryo and endosperm where measurements were made the coverings of the Sudan grass caryopses were thicker than those of Johnson grass caryopses. They were thicker also in light-colored than in dark-colored Sudan grass caryopses, this being the result of a looser arrangement of the different layers, especially the starch-bearing layer, of the pericarp and a less contracted condition of the inner integument in the light-colored caryopses. Only in the very darkest Sudan grass caryopses were the coverings structures as compact as in Johnson grass caryopses.

The tendency for the pericarp tissues to fall loosely apart along the layers betweeen the outer and inner epidermises was greater in sections of Sudan grass caryopses than in sections of Johnson grass caryopses.

RESISTANCE OF SEED COVERINGS TO RUPTURE FROM HEATING IN WATER, AND TO IMBIBITION BY THE EMBRYO

The differences in compactness of the coverings of Johnson grass and Sudan grass caryopses suggested that differences in mechanical resistance to rupture from the pressure of the expanding embryos might exist and might be significant in relation to the germination behavior of the caryopses. In an attempt to test the mechanical resistance of these

coverings, 100 each of the light-colored and dark-colored Sudan grass caryopses and of large and small Johnson grass caryopses were submerged in water in air-dry condition and slowly heated for three 20-minute periods with slow cooling between the periods of heating. The rate of heating was such that the water was just beginning to boil at the end of 20 minutes.

Scarcely any caryopses broke during the first priod of heating, but all but a very few had broken by the end of the third period. The majority broke first over the endosperm. At the end of the third period of heating 42 small and 26 large Johnson grass caryopses and 9 dark and no light Sudan grass caryopses had broken over the embryo. The breaking of the coat structures of Johnson grass over the embryo and the failure of these to break in Sudan grass caryopses is evidently the result of their looser arrangement in the latter case and not of differences in resistance to pressure from within. The looser coverings of the Sudan grass embryo, and especially of those of the light-colored caryopses, allowed greater swelling of the embryos before these coverings were distended to their full capacity and at the same time gave better opportunity for the escape of gases. A natural corollary of this fact is that, under germination conditions, the Sudan grass enbryo can imbibe a greater percentage of water without breaking the coverings than can Johnson grass embryos. If the force of imbibition of a Johnson grass embryo is insufficient to overcome the resistance of coat structures, the embryo will remain dormant on account of incomplete satisfaction of its water requirements. As a matter of fact actual tests with thick sections made with a freezing microtome and at once immersed in different solutions and examined with a microscope indicate that dormant Johnson grass embryos, even after long incubation under germination conditions, are about in equilibrium with 2-molar salt solution. The sections of the embryos contracted considerably in 4-molar salt solution and swelled very appreciably in water but underwent no appreciable change in 2-molar salt solution. Coat restrictions to water intake may, therefore, be important in imposing dormancy and resistance to germination here as with the seeds of many water plants (11, 12.)

COMPOSITION OF PERICARP AND INNER INTEGUMENT OF JOHNSON GRASS AND SUDAN GRASS CARYOPSES

Only a few microchemical tests were made by the authors. The results of these tests were verified and additional tests were made by Dr. Sophia Eckerson, of the University of Chicago and the Bureau of Plant Industry of the United States Department of Agriculture, for whose generous assistance we are greatly indebted. Table II summarizes Doctor Eckerson's results so far as they are significant in the present comparison.

The cell walls of the aleurone layer were of hemicellulose in both kinds of caryopsis. Although these walls are comparatively thick, it does not seem possible, in view of their composition and the much more resistant character of the integument and pericarp, to attribute to the aleurone layer any special protective function in uninjured caryopses.

The pericarp tissues contained the same elements in both kinds of caryopsis, except iron, which was present in the outer epidermis of Johnson grass and absent in that of Sudan grass, but pectic substances were much more abundant and the suberization was less in Sudan grass

than in Johnson grass. The composition of the inner integument was quite different in the two kinds of caryopsis. In Sudan grass caryopses it consisted mainly of hemicellulose, the inner walls being somewhat, and the outer walls rather more impregnated with suberin. In Johnson grass caryopses, on the contrary, the outer walls contained cellulose and were somewhat suberized, while the inner walls as well as the closing tissue were suberin, strongly impregnated with fatty substance. The amount of fat, both in the walls and in the cell contents, was much greater in Johnson grass than in Sudan grass.

In addition to the data shown in Table II, all layers of the pericarp and the integument contained tannin in both kinds of caryopsis, but more in Johnson grass than in Sudan grass.

TABLE II.—*Composition of coat structures of Johnson grass and Sudan grass caryopses*

Part examined.	Composition.	
	Johnson grass caryopses.	Sudan grass caryopses.
Aleurone layer.....	Cells walls hemicellulose.....	Cell walls hemicellulose.
Inner integument..	Inner cell walls pure suberin, impregnated with fatty substances; fat also in cell contents.	Inner cell walls hemicellulose, somewhat suberized; much less fat than in Johnson grass.
	Outer cell walls have very little suberin; some cellulose.	Outer cell walls hemicellulose, more suberized than inner cell walls.
Pericarp.....	All cell layers contain pectic substances and are suberized; a little calcium; a little cellulose; iron in outer layer.	Similar to Johnson grass but less suberized, with pectic substances much more abundant; no iron.
	All layers swell in 2 per cent oxalic acid and in ammonium hydrate of one-tenth commercial strength.	Swell much more than Johnson grass in oxalic acid and ammonium hydrate solutions.
"Closing tissue" of the hilar orifice.	Suberin and considerable oil, which comes out after 10 to 15 minutes' warming in alcohol.	Not determined.

COMPARATIVE RATES OF BLEACHING WITH JAVELLE WATER—CORRELATION WITH GERMINATION

These tests were made with one lot of Sudan grass caryopses and two lots of Johnson grass caryopses, using only uninjured caryopses which had been removed from the scales by hand. The Johnson grass seed was nearly two years old and was therefore fully after-ripened. When fresh, No. 37001 was an unusually ready germinator, but the San Antonio lot was exceptionally resistant to germination. Almost 30 per cent of the naked caryopses of the former and less than 5 per cent of those of the latter germinated in 10 days at 25° C. At other temperatures the comparison was similar. The caryopses were placed in small vials, and several times their volume of Javelle water was added. The Javelle water was renewed after 1, 2, 6, and 24 hours and at intervals of 24 hours thereafter until bleaching was complete. The first visible effect of the Javelle water was a pronounced darkening of the caryopses, probably due either to the action of the free alkali in the solution or to oxidations.

Caryopses of Sudan grass and Johnson grass No. 37001 became almost black in one-half hour, and those of the San Antonio lot in an hour. The initial blackening was followed by gradual bleaching, which was first apparent and proceeded most rapidly over the embryos and thence advanced around the endosperm at the proximal end, and at the same time toward the distal end over the entire circumference of the caryopsis. As the seeds whitened the bleaching solution darkened. More rapid loss of color in spots frequently gave a mottled appearance to the bleaching caryopses. The closing tissue of the hilar orifice and the inner walls of the very large integument cells at the distal end of the caryopses were the slowest to show the effect of the bleaching and the last to become completely bleached. Often the circular closing tissue of the hilum remained very dark 24 hours after all other tissue at the proximal end of the caryopsis was completely bleached.

In many Johnson grass caryopses the coverings over the edges of the scutellum and over the axial organs bleached more quickly than a little to either side of the axis, leaving two prominent dark lines the whole length of the embryo after the rest was white.

Table III shows in detail the progress of bleaching in the three lots of caryopses and the results of germination tests of the same lots of caryopses. The germination tests were made in 100 mm. Petri dishes with wet blotting paper as germination bed. Sudan grass was tested at room temperature and Johnson grass in an incubator at 26° C.

Examination of the data in Table III shows that Sudan grass caryopses bleached much more rapidly than Johnson grass caryopses and Johnson grass No. 37001 more rapidly than the San Antonio lot. Correlated with the difference in resistance of the two lots of Johnson grass caryopses to the action of Javelle water was a difference in the readiness with which they germinated. This latter difference, while only slight in the fully after-ripened condition at the time these tests were made, was, as previously indicated, very much greater when the seeds were fresh. Viability tests made after the third day by scratching the embryos along one side of the scutellum with a bent needle and returning them to the incubator for another day showed all the caryopses to be viable and capable of producing vigorous seedlings. The application of this method of determining the viability of Johnson grass embryos has been described elsewhere (15).

A few of the most resistant Sudan grass caryopses bleached more slowly than a few of the least resistant Johnson grass caryopses. It is perhaps worthy of notice also in this connection that 1 per cent of the Sudan grass caryopses, though viable and potentially vigorous, did not germinate until after the coverings of the embryo had been opened—a process which induces the germination of the most resistant Johnson grass caryopses even before they have after-ripened.

Additional bleaching tests were made with the San Antonio lot of Johnson grass, using caryopses which had failed to germinate in three days at 26° C. in comparison with others which had for three days been incubated as for germination except that germination was prevented by keeping them in an ice box. The caryopses remaining from the germination test bleached on the average more slowly than those which had been incubated in the ice box, so that none germinated. This seems to indicate that the caryopses of this lot which had germinated were in general those which would have bleached most rapidly in Javelle water.

TABLE III.—*Bleaching with Javelle water and germination of Sudan grass and Johnson grass caryopses*[1]

	Sudan grass.	Johnson grass No. 37001.	Johnson grass, San Antonio, 1916.
First evidence of bleaching.	60 seconds, lighter over embryo.	15 minutes, lighter patches over embryo.	15 minutes less than in No. 37001.
First coloring of Javelle water.	60 seconds.	30 minutes.	60 minutes barely perceptible.
First "axis" completely bleached.	45 minutes.	45 minutes.	120 minutes.
First embryo completely bleached.	75 minutes.	120 minutes.	None in 6 hours; some nearly so.
First seed completely bleached except closing tissue.	3 hours, several in 6 hours.	None in 6 hours.	None in 6 hours.
After 24 hours:			
Completely bleached.	17 per cent.	5 per cent.	1 per cent.
Nearly all bleached.	78 per cent.	6 per cent.	2 per cent.
Little more than one-half bleached.	5 per cent.	68 per cent.	8 per cent.
About one-half bleached.	0 per cent.	19 per cent.	10 per cent.
Less than one-half bleached.	0 per cent.	2 per cent.	79 per cent.
Embryos bleached.	100 per cent.	98 per cent.	26 per cent.
All but distal end bleached.	0 per cent.	10 per cent.	8 per cent.
All but closing tissue bleached.	0 per cent.	6 per cent.	0 per cent.
All but distal end and closing tissue bleached.	0 per cent.	58 per cent.	0 per cent.
Flat side bleached more than embryo.	0 per cent.	0 per cent.	24 per cent.
After 48 hours.	Completely bleached except small spots on a few caryopses.	All but 10 completely bleached or nearly so.	Less advanced than No. 37001. Seven only about one-half bleached.
After 72 hours.	Small spot on one caryopsis unbleached.	5 or 6 not completely bleached at distal end.	15 or 20 not completely bleached.
After 96 hours.	All completely bleached.	One not completely bleached.	One not completely bleached.
Germination:			
1 day.	98 per cent.	65 per cent.	44 per cent.
2 days.	1 per cent.	3 per cent.	19 per cent.
3 days.			
4 days[2].	1 per cent.	32 per cent.	36 per cent.
Percentage viable.	100.	100.	99.

[1] 100 seeds were used in each test, both for bleaching and germination.
[2] Caryopses scratched at end of third day with a bent needle to induce germination.

RESISTANCE OF COAT STRUCTURES TO THE ACTION OF CHROMIC ACID: CORRELATION WITH GERMINATION

The resistance of the inner integument and the closing tissue in Johnson grass caryopses to the action of chromic acid has already been referred to and might be inferred from the fact that these consist wholly or largely of suberin.

The first treatments were with halved caryopses of three lots of fully after-ripened Johnson grass seed showing 100 per cent viability in comparison with halved caryopses of dark-colored and light-colored Sudan grass seeds. The caryopses, either in air-dry condition or after soaking in water, were cut in halves along a median sagittal plane with a sharp scalpel and were then immersed in 50 per cent chromic-acid solution, which was frequently changed. At the end of 24 hours' soaking in the chromic-acid solution some of the halved caryopses were washed in water and examined with the microscope. At this time considerable endosperm, white and normal in appearance, remained within the half-shells, but the majority of the embryos were entirely disintegrated. The coverings over the Sudan grass embryos were very considerably fragmented, and in most cases the remaining tissue was fragile and soft and tended to collapse into the empty embryo cavity. In the great majority of the Johnson grass half caryopses, on the contrary, the shells over the embryo cavity were intact or only slightly fragmented and remained stiff and brittle. Many of these were kept several days longer in 50 per cent chromic-acid solution without undergoing entire dissolution of the enbryo coverings. Embryo coverings of caryopses of the Johnson grass lot which was most resistant to germination, especially before after-ripening, were more resistant to the action of the chromic-acid solution than embryo coverings of the other two lots.

Entire caryopses of these same lots of Johnson grass and Sudan grass and wheat grains from a lot showing 99 per cent germination in two days were next treated with 50 per cent chromic-acid solution in small vials each containing 100 caryopses. At 24-hour intervals the caryopses in the different vials were removed from the chromic-acid solution, thoroughly washed with sodium-bicarbonate solution followed by distilled water, and put to germinate. At the same intervals caryopses were withdrawn from another vial of chromic-acid solution for sectioning and microscopic examination.

All wheat embryos were uncovered and killed by the action of the chromic acid by the end of the first 24-hour period, though in the most resistant grains scutella and the larger part of the axial organs were still intact. Fragments of the outer coverings, sometimes including even the outer epidermis of the pericarp, remained.

With Sudan grass caryopses only the inner integument and the closing tissue of the most resistant individuals remained intact at the end of 24 hours' treatment. Occasional adhering remains of the outer coverings usually represented only the inner epidermis of the pericarp. The whole proximal end, both embryo and endosperm, tended to become slightly stained, showing the slight penetration of the acid. Only 1 per cent of the embryos of dark-colored caryopses were very weakly viable after 24 hours in the chromic-acid solution. Sixty-seven per cent of the embryos of dark-colored caryopses were wholly or partly disintegrated, while the other 33 per cent were not yet exposed by the disintegration of the inner integument. Light-colored caryopses were much less resistant than dark-colored caryopses, all embryos being more or less disintegrated and only a small distal portion of the endosperm remaining intact in several caryopses.

TABLE IV.—*Resistance of the coverings of Johnson grass caryopses to the action of 50 per cent chromic acid* [1]

Seed lot 37001.	Effect of varying length (days) of treatment with chromic acid.				
	0	1	2	3	5
Condition of caryopses:					
Apparently not affected, or slightly darkened or mottled over embryo	*Per ct.*	*Per ct.* 45	*Per ct.* 12	*Per ct.* 7	*Per ct.* 2
Portions of embryo or its coverings (especially axial organs) more or less darkened or many after more than 1 or 2 days' treatment with part of embryo eaten away	.....	50	83	93	98
Parts of endosperm eaten away through weak place in covering on flat side, usually opposite the embryo	.....	5	5		
Germination percentage at 26° C.:					
1 day	65	40	12	3	.....
2 days	3	4	2	3	0
3 days	0	0	1	.....	.....
Total germination after unscratched period [2]	68	44	15	6	0
3 days [2]	.....	.....	.....	0	0
4 days [2]	32	1	0	0	0
6 days [2]	0	0	0	0	0
Total viable	100	45	15	6	0

Seed lot from San Antonio, 1917.	Effect of varying length (days) of treatment with chromic acid.				
	0	1	2	3	5
Condition of caryopses:					
Apparently not affected, or slightly darkened or mottled over embryo	*Per ct.*	*Per ct.* 65	*Per ct.* 26	*Per ct.* 22	*Per ct.* 6
Portions of embryo or its coverings (especially axial organs) more or less darkened or many after more than 1 or 2 days treatment with part of embryo eaten away	.....	34	73	78	94
Parts of endosperm eaten away through weak place in covering on flat side, usually opposite the embryo	.....	1	1		
Germination percentage at 26° C.:					
1 day	48	44	15	8	.....
2 days	11	16	6	7	4
3 days	1	1	2	.....	.....
Total germination after unscratched period [2]	60	61	23	15	4
3 days [2]	.....	.....	.....	2	0
4 days [2]	40	2	1	0	0
6 days [2]	0	0	0	0	0
Total viable	100	63	24	17	4

TABLE IV.—*Resistance of the coverings of Johnson grass caryopses to the action of 50 per cent chromic acid*—Continued

Seed lot from San Antonio, 1916.	Effect of varying length (days) of treatment with chromic acid.					
	0	1	2	3	5	7
Condition of caryopses:						
Apparently not affected, or slightly darkened or mottled over embryo	*Per ct.*	*Per ct.* 73	*Per ct.* 50	*Per ct.* 24	*Per ct.* 7	*Per ct.* .....
Portions of embryo or its coverings (especially axial organs) more or less darkened or many after more than 1 or 2 days' treatment with part of embryo eaten away	.....	26	50	76	93	.....
Parts of endosperm eaten away through weak place in covering on flat side, usually opposite the embryo	.....	1	0			
Germination percentage at 26° C.:						
1 day	44	60	40	12	.....	.....
2 days	19	12	5	10	2	.....
3 days	0	0	1	.....	.....	.....
Total germination after unscratched period [2]	63	72	46	22	2	.....
3 days [2]	.....	.....	.....	4	0	1
4 days [2]	36	1	0	0	0	0
6 days [2]	0	2	0	0	0	0
Total viable	99	75	46	26	2	1

[1] The different lots are arranged in the order of increasing difficulty of germination, especially before after-ripening.
[2] Embryos scratched at end of second or third day with a bent needle to induce germination.

Table IV shows the main features of the result with Johnson grass caryopses. These were remarkably resistant to the action of the chromic acid. The following points deserve special mention:

1. The resistance of the different lots to the action of the acid increased with increasing difficulty of germination, the readiest germinator (No. 37001) being most rapidly attacked by the acid and the poorest germinator (San Antonio, 1916) being most resistant.

2. The action of the acid for 24 hours or a longer period so reduced the resistance of the coat structures that practically all the embryos which were still viable germinated in 2 or 3 days without "scratching" whereas about 40 per cent of the controls failed to germinate until after scratching. In the most resistant lot (San Antonio, 1916) the percentage which germinated without scratching was actually increased by 24 hours' treatment. This recalls the action of concentrated sulphuric acid in removing the embryo coverings and thus inducing prompt and complete germination (*15*). In the case of the chromic acid the least resistant caryopses were killed within the first 24 hours, but a larger number of the most resistant caryopses were rendered easily germinable by the action of the acid.

3. The percentage of viable caryopses, and after the first 24 hours the percentage which germinated without scratching, decreased progressively with increased length of time in the chromic-acid solution.

4. In contrast to wheat and Sudan grass caryopses, which were killed within 24 hours, a small percentage of Johnson caryopses were still viable after 5 to 7 days.

A few Johnson grass caryopses had small, less resistant areas in the coat structures on the flat endosperm side opposite the embryo. The chromic acid, penetrating through these areas, ate small, deep holes into the endosperm. Such caryopses usually germinated if the integument over the embryo was still intact and the embryo itself uncolored by the acid. In fact even the starchy endosperm tissue, on account of its very hard, compact, glassy texture, seemed to be considerably more resistant to the corrosive action and to the penetration of the acid than was true of Sudan grass or wheat endosperms.

Nearly all caryopses which germinated after treatment with chromic acid belonged to the first class shown in Table IV "apparently not affected." Of these nearly all produced strong vigorous seedlings when the length of the treatment did not exceed two days, with progressively weaker seedlings after longer treatments. All treated caryopses which germinated only after scratching produced very weak seedlings, while untreated caryopses which were brought to germination by scratching produced as vigorous seedlings as those which germinated without scratching.

Microscopic examination of sections of the most resistant caryopses showed the inner integument and the closing tissue of the hilar orifice intact even after seven days treatment. Frequently the outer walls of the integument cells after more than two days' treatment were thoroughly disintegrated in places or so weakened and brittle that they broke away in sectioning, leaving only the thick inner walls covering the aleurone. All pericarp layers were present in places except after the very longest treatments, but these were greatly attacked at the hilar orifice, micropyle, and style, usually laying bare the closing tissue and sometimes also the micropyle within 24 hours. In most of the less resistant caryopses small portions of the integument were eaten away by the acid before coloring or disintegration of the underlying tissue began, but in a few of the less resistant and several of the more resistant caryopses there was evidences of local penetration of the acid sufficient to cause discoloration, through intact portions of the integument. The integument cells were on the average much more quickly destroyed over the embryo than elsewhere, so that after several days' treatment the embryo coverings in many caryopses were largely gone and the embryos themselves entirely gone, leaving at the proximal end of the caryopsis only the integument cells on the flat side and the closing tissue of the hilar orifice. From such caryopses were obtained the integument portions from which figures 8 and 9 were drawn.

RESISTANCE TO PENETRATION OF IODIN SOLUTION

Brown, working with cereals (*3*) was the first to discover the existence of a nonliving, semi-permeable membrane surrounding any seed. He soon discovered (*3, 4*) that this membrane had selective qualities, allowing many solutes to pass through it readily, others only very slowly, and excluding still others. Schroeder (*28*) discovered a similar semi-permeable membrane in wheat grains, and showed that this membrane admitted both water and penetrant solutes mostly around the embryo and little if at all at the distal end of the grain. Collins (*9*) confirmed for barley Schroeder's result with wheat. His results led him to believe that the entry of both water and solutes took place almost wholly through the micropyle, which, however, he was unable to locate exactly. All of these authors agree that iodin passes through the selective permeable

membrane in question more readily than most solutes, but Collins concludes that the barley grain does not appear to possess perfect impermeability to any solute.

To test the resistance of the coat structures of Johnson grass caryopses to the penetration of iodin, caryopses of the lot which had proved to be most resistant to the action of chromic acid and of Javelle water (San Antonio, 1916, Table III and IV) were immersed in a very dilute iodin potassium iodid (I_2KI) solution for three days and then tested for germination or sectioned for microscopic examination. Since both Johnson grass and Sudan grass caryopses usually contain starch in the embryos, the penetration of iodin solution in these can be observed to better advantage than in the cereals with their starchless embryos.

About 4 per cent germinated very weakly after scratching of the embryo with a needle. In these the needle wound healed, with darkening of the surrounding cells, much as in normal untreated caryopses. In all of these germinable caryopses the iodin had penetrated the coat structures and stained the underlying starch, either locally in small patches of the endosperm, or less frequently around the periphery of nearly the entire endosperm; but in none of them was any of the starch in the embryo stained. In some of them small areas of the embryo or of the endosperm portions underlying the scutellum stained pinkish brown, possibly from the penetration of iodin unaccompanied by the potassium ions which are necessary for the formation of the blue starch-iodin combination.

In the majority of the caryopses which failed to germinate, both of this lot and of No. 37001 treated at another time both in air-dry condition and after soaking in water, the iodin entered and stained the starch most promptly and abundantly along the margins of the scutellum, staining both endosperm and embryo starch. The stained areas gradually spread to cover the whole adjoining surfaces of the endosperm and the scutellum. From thence the stained areas advanced to the central organs of the embryo, and along the periphery of the endosperm from the under surface of the scutellum toward the distal end of the caryopsis, thus paralleling in general the course described by Collins for barley. The evidence seems to indicate that the iodin entered most readily through the hilar orifice, or the micropyle, or both, passing at once around the caryopsis and in a distal direction along the inner surfaces of the aleurone layer and of the scutellum. The passage of the iodin to the middle part of the proximal end of the scutellum and to the adjoining starchy endosperm cells seemed to be retarded by the intervening large, empty endosperm cells lying between the hilar orifice, mircopyle, and proximal end of the scutellum, so that the distal end of the scutellum, though farther removed from the hilar region, became stained sooner than the proximal end. There were frequent undoubted cases also of local penetration of the iodin in some abundance through the uninjured integument. The iodin always passed only very slowly toward the center of the endosperm in a radial direction. In the periphery of the endosperm the extreme distal portion was almost invariably the last to stain. The penetration even around the embryo was not rapid enough to prevent the germination while in the iodin solution of about 16 per cent of one lot of caryopses which had previously been soaked in water in an icebox. Of course germination proceeded only to the extent of breaking the coverings over the embryo, after which the embryos were promptly killed.

Sudan grass, wheat, and dent corn caryopses were soaked in weak I_2KI solution and examined for comparison with Johnson grass caryopses.

Only uninjured caryopses were used. In Sudan grass caryopses the iodin followed the same course as in Johnson grass caryopses but penetrated at the proximal end nearly twice as rapidly. In all of the wheat grains black bands appeared round at least a part of the base of the embryo within 2 hours and spread within 24 hours around and under the embryos and a half or more of the way to the distal end of the grains. There were also local areas of penetration elsewhere in a few grains. All embryos at the same time became stained light brick red. In the corn, black areas appeared within a very short time, first at the dent then generally at various points scattered over the grain. At the end of 24 hours a thin starch-stained zone just within the coat structures covered the entire grains except the thick hull at the extreme proximal end and sometimes a portion of the dent.

It is evident, therefore, that even the easily penetrating iodin solution found access to the embryos of Johnson grass somewhat more difficult than to those of Sudan grass and much more difficult than to those of wheat and corn.

PENETRATION OF OTHER SOLUTES; POSSIBLE EFFECT UPON GERMINATION

In 2-molar and 4-molar solutions of sodium chloride, freshly harvested Johnson grass caryopses behaved at first as if they were protected by semipermeable membranes, imbibing quantities of water which decreased with increasing concentration of the solutions, losing water when transferred from the weaker solution with which they were in equilibrium to the stronger solution, and taking up water until they regained their previous weight when the reverse change was made. For some time they retained their viability when thinly covered with the salt solution, but by the end of several weeks they had taken up sufficient salt to kill all of the embryos.

Immersion of the freshly harvested caryopses for seven days in molar solutions of potassium sulphocyanid (KCNS), sodium sulphocyanid (NaCNS), and lithium nitrate ($LiNO_3$) killed all of the caryopses; nearly all were killed by immersion for seven days in molar solutions of potassium nitrate (KNO_3), sodium chlorate ($NaClO_3$), barium sulphocyanate ($Ba(CNS)_2$), and urea ($CO(NH_2)_2$); about one-half survived seven days' immersion in molar solutions of lithium suplhate (Li_2SO_4), barium nitrate ($Ba(NO_3)_2$), magnesium nitrate ($Mg(NO_3)_2$), and potassium tartrate ($K_2C_4H_4O_6$). Molar solutions of other salts for the same length of time caused less injury, and immersion in water for seven days caused no loss of viability.

Immersion of fully after-ripened caryopses for one or two days in 5 per cent acetic acid (CH_3COOH), 5 per cent hydrochloric acid (HCl), 5 per cent ammonium hydroxid (NH_4OH), 3 per cent alcoholic potassium hydroxid (KOH), 95 per cent alcohol, acetone, ether, chloroform, or xylol killed all of the caryopses; immersion for one day in 3 per cent sulphuric acid (H_2SO_4) killed 80 per cent of them; and immersion for two days in saturated calcium chlorid ($CaCl_2$) solution killed 20 per cent of them. The controls soaked two days in water, were uninjured. All of the solvents or solutes mentioned in this paragraph increased the subsequent rate of bleaching of the thoroughly washed caryopses with Javelle water and the rate of penetration of iodin solution; hydrates had the most effect, fat solvents next, followed by acids and by ($CaCl_2$), which had very little effect.

COAT CHARACTERS IN RELATION TO GERMINATION

It can be seen from the preceding section that many solutes of various chemical nature pass through the membranes covering Johnson grass embryos. It has also been shown that certain chemical treatments greatly favor germination. These treatments include removal of the coverings over the embryo by means of concentrated sulphuric acid, treatment with chromic acid for a long enough time to weaken these coverings without killing the embryo, treatment with mercury salts, subjection to atmospheres with high concentration of carbon dioxide, and etherization (*15*, *16*). Furthermore, certain of the salts mentioned in the preceeding section which were toxic in molar solutions slightly stimulated germination when used in tenth to hundred thousandth molar solutions and hydrogen peroxid in proper concentrations is also an efficient forcing agent.

The question arises: Do the beneficial effects of these chemical treatments result from stimulation of the embryo protoplasm or from the removal or lessening of coat restrictions? If the latter they may produce the effects observed either by increasing the permeability of the coat structures to solutes, thus admitting oxygen or releasing inhibitors which are held by them, or by decreasing the mechanical resistance of the coat structures to the expansion of the embryo.

Denny (*13*) has shown that tannins, lipoids, and pectic substances greatly decrease the permeability to water of seed coats which are impregnated with them, while suberized membranes were not significant in the seeds which he studied except as these membranes became impregnated with fatty substances, which he showed did decrease their permeability.

The pectic substances and hemicellulose in the coat structures of Sudan grass caryopses including the inner integument indicate that these membranes would probably take up water slowly, but in larger total amount than those of Johnson grass, thereby becoming more distended, with a greater weakening of their mechanical resistance. The greater abundance of tannin and of suberin and its associated fatty substances in the coat structures of Johnson grass, on the other hand, would tend to limit the total amount of water which they are able to absorb and their permeability to water and to substances in aqueous solution below the level obtaining for Sudan grass but should not effect the total amount of water which might in course of time pass through them if the embryo and endosperm were able to absorb it. These limitations would reach their maximum in the inner walls of the inner integument and in the closing tissue of the hilar orifice.

Johnson grass caryopses, either in the scales or with the scales removed, take up water so rapidly that limited permeability of their coverings to water can not be considered as a possible cause of their dormancy and germination physiology. In fact the freshly harvested, dormant, naked caryopses absorb water when immersed in it so that their total moisture content is about 50 per cent of their dry weight at the end of 24 hours, after which small amounts are absorbed. So far as water intake is concerned, therefore, the coat structures need be considered only as possibly limiting by their physical resistance, the total amount of water imbibed by the caryopses.

It is probable, however, that the substances which Denny (*13*) found limiting the permeability of membranes to water would also decrease

their permeability to substances in aqueous solution. We have shown that this is true so far as the effect of lipoid substances upon the penetration of I_2KI solution is concerned. If on account of restricted permeability metabolically developed inhibitors to germination were prevented from escaping from the embryo as suggested by Kidd (*20*) and by Mazé (*23*, *24*), or if the concentration of oxygen within the embryo were thus maintained below minimum required for germination, dormancy would result.

It has been shown (*16*) that carbon dioxid in a wide range of concentration forces the germination of dormant Johnson grass caryopses, showing that Kidd's earlier hypothesis which indeed he and West (*21*) modified in a latter paper, does not apply in this case. We have found, furthermore, that increased partial pressures of oxygen in the atmosphere or even very high oxygen pressures are not effective in forcing the germination of Johnson grass. Limited oxygen supply, therefore, does not seem to play the rôle here that has been shown for dormant wild oats (*1*) Xanthium (*10*, *29*) and other seeds; the forcing action of hydrogen peroxid on the germination of Johnson grass must apparently have some other explanation than increasing the oxygen supply of the embryo.

As for Mazé's hypothesis of acetic aldehyd as an inhibitor to germination Brown (*4*), Schroeder (*28*), Collins (*9*), and Brown and Tinker (*5*, *6*) have shown that acetic aldehyd and smilar compounds pass through the selective permeable membranes of wheat and barley rather readily. In our own work ether, acetone, chloroform, 95 per cent alcohol, and Xylol all entered Johnson grass caryopses in 24 hours at room temperature in sufficient quantity to kill the embryos. It is highly improbable, therefore, that acetic aldehyd would be kept in by the coats in sufficient concentration to hold the caryopses in a dormant condition for months or years under good conditions of moisture and aeration, as we have found to be the case with Johnson grass caryopses. The force of this argument is increased when we consider that if acetic aldehyd is present in the caryopses it is there as a product of respiratory activities, and that respiration is on a very low level in the dormant caryopses. If there were other possible water-soluble inhibitors present in the embryos, the same logic would apply to their removal.

If we turn now to the possible explanation of coat effects as related to the swelling of the embryo, we find the following situation: Sulphuric acid removes the coat structures; chromic acid weakens them; mercury salts probably tend to coagulate the coat colloids and may thus weaken the coat structures; lipoid solvents dissolve a part at least of the lipoid substances with which they are impregnated, thus increasing their permeability and probably also weakening their physical resistence; and salts other than those of mercury which increase germination (chlorates, sulphates, nitrates, sulphyocyanates) tend to increase the hydration of colloids and may thus weaken the coat structures. All these substances might be supposed to produce their beneficial effects upon germination, in part at least, by altering coat colloids. This can hardly be the case, however, with carbon dioxide, which probably passes through the pericarp and integument in solution and acts upon the embryo itself. Undoubtedly also all of the other substances which have been shown to stimulate germination pass through the covering membranes of the caryopses at least in very limited amounts. These membranes apparently

are not completely impermeable to any solute. These substances, even the highly toxic salts of mercury, may therefore reach the embryo in exceedingly small, subtoxic or only slightly toxic doses—much weaker than the solutions in which the caryopses are soaked—and stimulate it into growth. Moreover, treatment of freshly harvested Johnson grass caryopses with tenth molar to hundred thousandth molar solutions of hydrochloric, acetic, oxalic, citric, and tartaric acids and of sodium hydrate—all of which are colloid hydrators—did not increase their germination as we should expect if decreasing the coat resistance by increasing the hydration of coat colloids were the only factor involved.

Apparently with chromic acid the treatments which stimulate germination are such as just fall short of serious injury, exactly as Kidd and West (*21*) reported for dormant white mustard seed with a number of stimulating agents, which are not classifiable under any other head. Also some at least of the other substances which increase germination are toxic to the intact, dormant caryopses if employed in too great concentration. In the case of the wounding of the scutellum with a needle there is always definite, concrete evidence of a reaction of the living protoplasm in the prompt darkening (probably from suberization) of the cell walls along the wound surfaces. This reaction takes place only slowly if at all in dead embryos when these are scratched.

The argument set forth in the preceding paragraph might lead to the hypothesis that the favoring effect of various treatments upon germination is entirely the result of stimulation of the embryo protoplasm. This hypothesis, however, leaves unexplained one important earlier observation (*15*). If the distal ends of the dormant caryopses are cut off just back of the ends of the embryos and the embryo portions are put to germinate, the following reactions ensue. First, a slight distension of the starchy endosperm beyond the edges of the cut surfaces of the coat structures; second, after a day or two the cells of the epithelial layer of the scutellum begin to elongate in the region of the cut surface and the digestion of the starch begins in the endosperm cells underlying such areas of elongation exactly as in the early stages of normal germination of inmutilated caryopses; third, a few days later, normal germination.

The most probable explanation of this set of phenomena is the effect of an increased swelling capacity of the embryo due to the reduction of the mechanical pressure upon it, this time working from behind the embryo and effecting it more slowly than when the coverings were removed from the embryo itself. To be sure, the radicle and epicotyl break through their coverings when germination occurs exactly as in the germination of unmutilated caryopses, but as the result of growth forces which are greater than the imbibitional force of the partially imbibed embryo, and which could not be initiated so long as the swelling of the embryo was greatly restricted. And it has been shown in a previous section (see p. 205) that the embryos of dormant imbibed caryopses are not completely satisfied with water. Theoretically increased oxygen supply to the embryo or the removal of an inhibitor to germination may play a part also, but the theory of increased imbibition seems much more plausible.

Cereals and other grasses which were not after-ripened have been induced to germinate by this method of cutting the grain in two just back of the embryo. Others (*2*, *18*) also have induced germination of cereals which were not after-ripened by wounding the endosperm, and have

attributed the result to effects upon oxygen or water absorption. To be sure, Kiessling (*22*) reported that coating the wounded endosperms with substances which he claims prevented increased imbitition as a result of the wounding did not prevent the stimulating effect upon germination and used this fact in support of his "stimulus" hypothesis. But it is difficult to accept this hypothesis, because the only avenue for the transmission of such a stimulus to the embryo through the medium of living cells appears to be along the aleurone layer, and even this path is interrupted along the adjoining faces of the scutellum and endosperm by a mass of compressed nonliving endospermic cell walls. Furthermore, in our experiments with Johnson grass caryopses the first evidences of vital and enzymic action were observed, not where the aleurone approaches the scutellum—that is, at the extreme distal end of the scutellum, but in the area nearest to the cut surface—that is, several epithelial cells from the extreme distal end.

There is one strong indication that the coat structures are more or less directly responsible for the dormancy and germination physiology of Johnson grass caryopses independently of any effect upon the embryo protoplasm of the operation of removing or weakening these structures, namely, the correlation which has been shown in previous sections between the resistance of these structures to certain reagents, on the one hand, and the germination of the fresh caryopses, on the other. That differences in these coat structure occur as between different kinds of seed with different germination requirements might be wholly incidental and irrelevant. But when in addition similar differences occurring in different lots of the same kind of seed parallel in a logical manner differences in germination under identical external conditions, the parallelism can hardly be without significance. These considerations suggest that the inhibitory effect of the unusually tough and compact coverings of the embryo may be related primarily to a purely physical restriction of the imbibitional swelling of the embryo, especially its axial organs, a restriction which is enhanced by the location of these organs almost wholly enveloped by the massive wings of the scutellum and is increased also by the pressure of the tight-fitting scales within which the caryopses are inclosed. That such a physical restriction is paralleled by a decrease in permeability of the coat structures and an increase in their resistance to chemical attack is entirely natural, since the tensile strength, elasticity, and extensibility of the coat structures is determined by the degree of drawing together of the individual elements and the degree of their impregnation with substances which are insoluble in water and which confer relative impermeability. It should be stated, however, that actual proof of this hypothesis, which relates the tardy germination of Johnson grass seeds to restricted imbibition by their embryo, is, in fact, wanting. As already pointed out, certain facts indicate that other factors, perhaps involving direct stimulation of the embryo protoplasm, enter into the forcing of their germination by chemical reagents. A sharp distinction should be maintained between mechanical and chemical forcing of germination even when, as here, the same kind of seeds is used in both cases. The fundamental explanation may be quite different in the two cases.

SUMMARY

(1) Johnson grass seed is markedly dormant when first matured under ordinary conditions of storage, requires a number of months for complete after-ripening, and even when fully after-ripened will not germinate completely except with the use of alternating temperatures in a very warm temperature range. Seeds of its close taxonomic relative, Sudan grass, germinate freely with a wide range of temperature either constant or alternating, and without the intervention of any considerable period of after-ripening.

(2) Johnson grass caryopses are invested in the fruit with very hard, tight, usually darkly pigmented scales (glumes), the removal of which accelerates the germination of the caryopses and increases their germinating capacity.

(3) The coverings of the naked mature caryopses of both grasses consist of the fused pericarp and inner integument, the outer integument and nucellus having entirely disappeared, with the possible exception of a portion of the latter over the micropyle. The complete or partial removal of these coverings over Johnson grass embryos induces prompt and complete germination even of freshly matured caryopses, and under the temperature conditions for the germination of Sudan grass seed.

(4) The pericarp of both kinds of caryopsis consists of a continuous outer epidermis, a fragmented inner epidermis, and several intermediate layers of loosely arranged cells. One of these layers frequently contains starch. The outer epidermis does not have especially thick walls and is easily broken. The pericarp tissue breaks jaggedly at the pedicel when the caryopsis is removed from the scales, thus opening a passage for solutes into the pericarp tissue clear to the inner integument.

(5) The inner integument of both kinds of caryopses is highly developed and of large cells with very thick, darkly pigmented inner walls.

(6) The micropyle is usually completely closed by a massive, recurved development of the inner integument.

(7) The large circular hilar orifice contains no vascular elements, conduction from the vascular bundle of the pedicel and outer layers of the pericarp over the hilar region being by means of parenchymatous pericarp tissue which entirely fills the hilar orifice, and is fused with the inner integument at the hilar margins.

(8) A zone of this conducting pericarp tissue lying just outside the hilar orifice and including the elements which are fused with the inner integument becomes greatly contracted radially and darkly pigmented during the maturation of the caryopsis. This pigmented zone of the pericarp and the inner integument together constitute for the caryopsis an unbroken investment which is extremely resistant to the action of Javelle water and of chromic acid and has the quality of selective permeability, though it probably does not exclude any solute entirely. Penetrant solutes (iodin solution) enter much more readily at the proximal end of the caryopsis, probably through the hilar orifice, than elsewhere, but they also frequently enter locally in other places.

(9) The coverings of Sudan grass caryopses are more fragile and their embryos are less tightly inclosed and are so situated as to be more exposed to mechanical injury than is the case with Johnson grass caryopses.

(10) The coverings of the caryopses are thicker over the hilar and micropylar regions and in front of the point of the radicle in Johnson grass

caryopses than in Sudan grass caryopses, this difference being related to the more forward exposed position of the Sudan grass embryos. In other regions the coverings of Sudan grass caryopses are thicker than those of Johnson grass caryopses but are less compact and less darkly pigmented and offer less effective insulation to the embryo.

(11) The coverings of Johnson grass caryopses are much more resistant to the bleaching action of Javelle water and the corrosive action of chromic acid and are somewhat less readily penetrated by iodin than are those of Sudan grass caryopses. The lots of Johnson grass caryopses which are most resistant to the action of Javelle water and of chromic acid are most deeply dormant when fresh and most resistant to germination when after-ripened.

(12) The coverings of wheat caryopses are less resistant to the action of chromic acid than are those of Sudan grass caryopses.

(13) The coverings of wheat and corn are more readily permeable to iodin than are those of Sudan grass caryopses.

(14) Soaking Johnson grass caryopses in lipoid solvents kills the embryos and at the same time increases the rate of the subsequent penetration of iodin and the rate of subsequent bleaching in Javelle water.

(15) The inner integument and the various layers of the pericarp of Johnson grass all contain tannin compounds, and all are highly suberized, especially the inner wall of the inner integument, which consists of suberin impregnated with fats and to which is due the great resistance of the caryopses to the action of chromic acid. Probably tannin, suberin, and lipoids all increase the strength and diminish the extensibility of the coat membranes and decrease their permeability to solutes, and probably all are related to the inhibiting effects of the coverings of the caryopsis upon germination.

(16) While this hypothesis is not subject to exact proof it seems probable that the character of the coverings of Johnson grass caryopses limits the imbibitional swelling of the embryos and thus keeps their water content below the minimum required for the inception of germination at relatively low and constant temperatures. Removal of the distal ends, exposing the caryopses to increased imbibition, induces enzymic activity in the scutellum followed by germination, reversing the order which characterizes normal germination. It is possible that inhibitory substances are held within the coats and that these maintain the embryo in a dormant condition. The effective forcing agents may oxidize or precipitate these substances, or they may modify the permeability of the coat structures so they can diffuse. Breaking the coat structures would also lead to the exit of such materials.

(17) Chemical treatments which increase the germination of Johnson grass and the wounding of the embryo may involve both a reduced resistance of the embryo coverings to imbibition and a direct stimulus to the embryo protoplasm.

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PROBABLE CAUSE OF THE TOXICITY OF THE SO-CALLED POISONOUS GREENSAND [1]

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PURPOSE OF THE INVESTIGATION

In an article by True and Geise [2] the authors mention a poisonous variety of greensand located at Courtland, Va. It was found that when greensand from this deposit was applied to plants, even in small quantities, the plants were unfavorably affected, while greensand from other deposits was beneficial.

Since the valuable potassium content of this so-called poisonous greensand is as high and as easily available as that found in the other deposits, it was thought advisable to make a more complete study of this variety with a view to discovering its poisonous constituents, and if possible to find some means of overcoming the unfavorable feature, thereby rendering useful this and other deposits that might show similar properties.

CHEMICAL ANALYSES OF TOXIC AND NONTOXIC GREENSAND

For the purpose of comparing the toxic greensand with the nontoxic greensands, chemical analyses were made of deposits from Redbank, N. J., and Newcastle, Va., in addition to that from Courtland. The material used for these analyses was obtained from the same deposits as those studied by True and Geise, and the analyses were made with great care. The results are given in Table I.

TABLE I.—*Analyses of greensand from New Jersey and Virginia*

Constituents.	Courtland, Va.	Newcastle, Va.	Red Bank, N. J.	Remarks.
	Per cent.	*Per cent.*	*Per cent.*	
SiO_2	60.14	39.83	56.07	Analysis made by using 20 per cent hydrochloric acid and digesting.
SO_3	.53	2.01	Absent.	
CaO	1.23	10.42	4.62	
MgO	4.10	1.74	1.80	
Mn_2O_3	.28	1.02	Trace.	
P_2O_5	Trace.	.54	.95	
Fe_2O_3	13.60	14.08	12.00	
FeO	7.56	4.89	8.28	
Al_2O_3	8.06	10.04	7.02	
Cr_2O_3	Strong trace.	Absent.	Absent.	
NiO	do	do	do	
Free sulphur	Present in appreciable quantity.	do	do	Free sulphur removed by successive extraction with ether and recrystallization.
K_2O	4.76	2.10	7.63	
Na_2O	.71	.49	.47	

[1] Accepted for publication July 2, 1921.

[2] TRUE, RODNEY H., and GEISE, FRED W. EXPERIMENTS ON THE VALUE OF GREENSAND AS A SOURCE OF POTASSIUM FOR PLANT CULTURE. *In* Jour. Agr. Research, v. 15, no. 9, p. 483-492, pl. 33-34. 1918.

An examination of Table I shows that the only elements found in the Courtland greensand which were not present in the other two were chromium, nickel, and sulphur, and it might therefore be concluded that these were responsible for the toxic action. Such, however, does not appear to be the case, since an analysis of 1,000 gm. of the Courtland greensand showed only 0.63 gm. chromium oxid, 0.2504 gm. nickel oxid, and 1.6 gm. free sulphur. It must be stated, however, that with the large percentage of iron and aluminum present it was difficult to estimate the small quantity of chromium and nickel, and a close approximation is all that is claimed for the figures here given. The part that free sulphur may play seems doubtful. Plants treated with a quantity of sulphur corresponding to that found in the toxic greensand, when mixed with the nontoxic greensands, failed to show any ill effects, yet it is possible that sulphur in intimate contact with some of the other elements concerned may be harmful. The Courtland greensand, however, was still toxic to plants after the free sulphur had been removed.

The ferrous and ferric salt content differed but slightly in the three deposits, while the manganese content was much higher in the nontoxic Newcastle greensand than in the other two, and the calcium salt much less in the Courtland variety than in either of the others.

EFFECT OF WATER-SOLUBLE SALTS OF GREENSAND ON GROWTH OF PLANTS

To determine which compounds were water-soluble, and thus available to plants, portions of each variety of greensand were suspended in distilled water, constantly stirred for 12 hours by a mechanical stirrer, and the solutions were filtered, concentrated, and analyzed. The results are given in Table II.

TABLE II.—*Analyses of washings of greensand from New Jersey and Virginia*

Constituents.	Courtland, Va.	Newcastle, Va.	Red Bank, N. J.
	Per cent.	*Per cent.*	*Per cent.*
SiO_2	60. 17	32. 83	44. 03
SO_3	2. 19	2. 01	5. 40
CaO	1. 52	18. 42	7. 70
MgO	2. 36	1. 74	1. 90
Mn_2O_3	1. 18	1. 02	. 52
P_2O_5	Trace.	. 54	. 94
Na_2O	1. 05	. 49	. 49
K_2O	2. 01	1. 10	1. 27
Organic or volatile substances	.....	9. 07	13. 91

Plants grown in these washings were healthy and vigorous for three weeks, after which it was observed that those in the Courtland washings began to lag behind, and in another week they were dead. It will be observed (Table II) that the washings from the Courtland greensand showed a greater solubility of manganese than the washings from either of the others, although the manganese content as shown in Table I was greatest in the Newcastle greensand. Table II shows also that the calcium is much less in the washings from the Courtland greensand than from either of the others.

From these data it was thought that the toxicity of the Courtland greensand might be due to manganese, iron, or aluminum in the absence of lime.

Since greensand is a mixture of glauconite, shell marl, and other adhering elements, it was desirable to determine the percentage of glauconite in each of the three deposits and test its action upon the growth of plants. The separations were made by washing off the light materials in the greensand. From the residue containing glauconite and other heavy materials the glauconite was separated in a relatively pure state by means of a magnet.[3] The washings were evaporated to dryness on the steam bath and dried to constant weight in an oven. The results are given in Table III.

TABLE III.—*Mechanical analyses of greensand from New Jersey and Virginia*

Source.	Weight of greensand airdried.	Glauconite.		Residue.
	Gm.	*Gm.*	*Per cent*	*Per cent.*
Courtland, Va.	62.3350	40.3420	64.72	35.28
Newcastle, Va.	36.8440	5.4028	14.6	85.40
Redbank, N. J.	41.8820	6.4290	15.0	85.00

From Table III it will be observed that the Courtland greensand contained much the highest percentage of glauconite.

The chemical composition of the samples of glauconite thus obtained and of the untreated toxic greensand is shown in Table IV.

TABLE IV.—*Comparative analyses of glauconite and toxic greensand*

Constituents.	Glauconite.			Toxic greensand.
	Courtland, Va.	Newcastle, Va.	Red Bank, N. J.	
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
SiO_2	51.52	49.46	48.26	60.14
SO_3	.04	.02	.02	.53
CaO	.72	.24	2.49	1.23
MgO	2.00	4.10	1.80	4.10
Mn_2O_3	.55	.64	.15	.28
P_2O_5	Trace.	.51	1.64	Trace.
Fe_2O_3	25.37	24.65	24.95	20.19
Al_2O_3	3.95	6.08	7.02	8.06
Cr_2O_3	Absent.	Absent.	Absent.	Trace.
NiO	do.	do.	do.	Do.
Free sulphur	do.	do.	do.	Do.
K_2O	6.29	7.10	7.08	4.76
Na_2O	.63	.56	.47	.71

Solutions were made from the various samples of glauconite by digesting with 20 per cent hydrochloric acid until digestion was complete, evaporating to dryness, heating to dull red heat, and dissolving the residue in dilute hydrochloric acid. These were made up to 500-cc. volume each.

Fifty cc. of each of these solutions were made neutral with ammonia and placed in a 250-cc. Erlenmeyer flask with sufficient distilled water

[3] ASHLEY, George H. NOTES ON THE GREENSAND DEPOSITS OF THE EASTERN UNITED STATES. *In* U. S. Geol. Survey Bul. 660, p. 27-49, fig. 1, pl. 2. 1918.

to make 250 cc. Paraffined corks, through which a small hole had been bored, were placed in the necks of the flasks. Corn seedlings were placed on the corks so that the roots passing through the holes were well covered with the liquid. At the end of 24 hours the seedlings in all the cultures were dead. The experiment was repeated three times with the same result, showing that the glauconite from each of these deposits was toxic.

In order to test the effect of the manganese, iron, and aluminum upon the growth of plants a series of experiments was conducted with corn seedlings in the following manner: A solution was made by extracting 3,000 gm. of the Courtland greensand by percolation for 72 hours with 3,000 cc. of 1 per cent hydrochloric acid. The percolate was evaporated to dryness and heated to dull red heat. The residue was dissolved in dilute hydrochloric acid and made up to 500 cc.

From 50 cc. of this solution manganese, iron, and aluminum were separated. The manganese was then separated from the iron and aluminum and added to the original filtrate. Similar solutions were prepared with iron and aluminum. The filtrate was then evaporated to dryness and the residue heated to dull red heat and dissolved in acidulated distilled water. The solution was made nearly neutral with a small quantity of ammonia and diluted to a volume of 250 cc. with distilled water.

From another 50 cc. of the solution, made up to 100 cc. with distilled water, the manganese, iron, and aluminum were all removed by precipitation with ammonia. The precipitate was discarded. The filtrate was evaporated to dryness and heated to a dull red heat until all the ammonium salts had been removed. The residue was then dissolved in distilled water acidulated with hydrochloric acid, neutralized with ammonia, and made up to 250 cc. with distilled water.

The effect of these solutions upon the growth of corn seedlings was tested in cultures in the manner described above. The results are given in Table V.

TABLE V.—*Comparative action on corn seedlings of various solutions prepared from Courtland greensand*

Number of days.	Manganese in solution (iron and aluminum removed).	Iron in solution (manganese and aluminum removed).	Aluminum in solution (iron and manganese removed.)	Soluble salts in solution (iron, manganese, and aluminum removed.)
1	Plant killed.....	Plant killed.....	Plant killed.....	Plant growing.
2	New plant started.	New plant started.	New plant started.	Do.
3	Plant killed.....	Plant killed.....	Plant killed.....	Do.
4	New plant started.	New plant started.	New plant started.	Do.
5	Plant killed.....	Plant killed.....	Plant killed.....	Do.
6	New plant started.	New plant started.	New plant started.	Do.
7	Plant killed.....	Plant-killed.....	Plant killed.....	Do.
8	.....	.....	.....	Do.
9	.....	.....	.....	Do.
10	.....	.....	.....	Do.

From Table V it will be observed that none of the seedlings survived in the solutions containing either the manganese, iron, or aluminum, while in the solution from which these had been removed they were still growing after 10 days.

EFFECT OF LIME ON ACTION OF TOXIC GREENSAND

In order to test also the effect of the toxic greensand upon the growth of plants when used in connection with an acid or an alkaline medium, the solutions described below were prepared.

A 3,000-gm. portion of Courtland greensand was extracted for 72 hours by percolation with 3,000 cc. of distilled water saturated with carbon dioxid. The percolate was evaporated to dryness, heated to a dull red heat, dissoved with very dilute hydrochloric acid, and made up to a volume of 500 cc. Of this solution 50 cc. were then made up to a volume of 250 cc. with distilled water. In this solution corn seedlings were grown in the manner already described.

Another 3,000-gm. portion of the same variety of greensand was extracted for 72 hours by percolation with 3,000 cc. of lime water. The filtrate was evaporated to dryness, heated to dull red heat, and dissolved in dilute hydrochloric acid. The solution was neutralized with a little ammonia and made up to a volume of 500 cc. Of this solution 50 cc. were then made up to 250-cc. volume with distilled water, and corn seedlings were placed in the solution as before.

The effect of these solutions upon the corn seedlings is shown in Table VI.

TABLE VI.—*Comparative action of solutions containing salts extracted with various solvents*

Number of days.	Solution containing salts extracted with carbon dioxid.	Solution containing salts extracted with lime water.
1	Plant killed	Plant growing.
2	New plant started	Do.
3	Plant killed	Do.
4	New plant started	Do.
5	Plant killed	Do.
6	New plant started	Do.
7	Plant killed	Do.
8		Do.
9		Do.
10		Do.

From Table VI it will be seen that the carbon-dioxid solution was toxic to the seedlings, whereas the plants in the alkaline solution were still growing after 10 days.

The results of analyses of the two percolates are given in Table VII.

TABLE VII.—*Analyses of acid and alkaline percolates*

Constituents.	Carbon-dioxid extraction.	Lime-water extraction.
	Per cent.	*Per cent.*
SO_3	16. 13	5. 90
CaO	7. 20	32. 00
MgO	3. 08	16. 20
Mn_2O_3	1. 16	. 80
P_2O_5	Trace.	Trace.
Fe_2O_3	3. 00	Absent.
Al_2O_3	Absent.	Do
K_2O	4. 86	7. 60
Na_2O	10. 86	28. 19

From these analyses of the solutions it will be observed that the lime-water extraction contained no iron or aluminum and only a very small quantity of manganese, while the potash was much higher in the lime-water extraction than in the carbon-dioxid solution. It can therefore be assumed that the carbon dioxid of the soil water, in connection with the acid secretion of the roots, would be amply sufficient to bring the toxic elements into solution and that a sufficient quantity of lime would inhibit this action.

Pot cultures were also made to test the effect of the toxic greensand, the soluble salts, the glauconite, and the greensand when used with the addition of lime. The accompanying illustrations show very strikingly the effect in each case.

Plate 1, A, shows corn seedlings 6 weeks old grown in quartz sand to which had been added glauconite from the Courtland greensand at the rate of 10 tons per acre, and Plate 1, B, shows the effect of the greensand with most of the glauconite removed.

Plate 2, A, shows the effect of the alkaline medium, principally salts of calcium, sodium, and potassium, from the washings of the Courtland greensand, at the rate of 3 tons per acre, and Plate 2, B, the effect of the heavy metals, principally magnesium, iron, aluminum, and manganese, obtained from the Courtland greensand by extracting with water made slightly acid with hydrochloric acid, but free from lime, applied at the rate of 2 tons per acre. To this there was also added a liberal application of Shive's nutrient solution.[4]

Plate 3 shows the effect of the toxic greensand when used alone and in combination with lime carbonate at the rate of 4 tons per acre.

A comparison of Plate 1, A, with Plate 2, B, and Plate 3, A, shows that the glauconite, heavy metals, and toxic greensand had practically the same toxic effect, while Plate 2, A, and Plate 3, B, show the very favorable effect of the addition of lime. It seems probable, therefore, that the toxic properties of this poisonous greensand may be overcome by using liberal applications of lime, thereby keeping out of solution the three poisonous elements, iron, aluminum, and manganese in toxic concentrations and rendering useful the potash which is readily available and which is greatly needed in many localities.

SUMMARY

The glauconite element from each of the three deposits of greensand studies was found to be toxic to corn seedlings.

The toxicity is due to the presence of iron, aluminum, and manganese in forms readily soluble in weakly acid media.

The presence of lime prevents the toxic elements from going into solution. Therefore, deposits of greensand like those at Newcastle, Va., and at Redbank, N. J., which naturally contain lime in the form of shell marl in sufficient quantity to prevent the solution of the toxic elements are not poisonous to plants. Likewise the addition of lime to the toxic Courtland greensand inhibits its toxic effects.

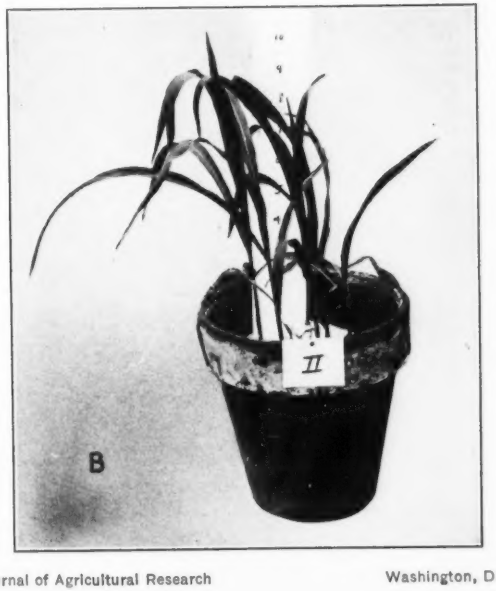
[4] SHIVE, JOHN W. A THREE-SALT NUTRIENT SOLUTION FOR PLANTS. *In* Amer. Jour. Bot., v. 2, no. 4, p. 157–160. 1915.

PLATE 1

A.—Corn seedlings 6 weeks old grown in quartz sand mixed with glauconite from Courtland greensand at the rate of 10 tons per acre.

B.—Corn seedlings 6 weeks old grown in quartz sand mixed with Courtland greensand from which most of the glauconite had been removed, at the rate of 10 tons per acre.

I
A


II
B

III
A

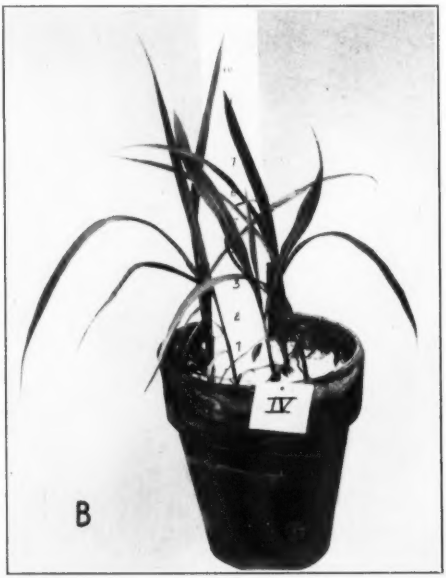

IV
B

PLATE 2

A.—Corn seedlings 6 weeks old grown in quartz sand mixed at the rate of 3 tons per acre with alkaline salts from washings of the Courtland greensand.

B.—Corn seedlings 6 weeks old grown in quartz sand mixed with the heavy metals obtained from the Courtland greensand, but free from lime, at the rate of 2 tons per acre.

PLATE 3

A.—Corn seedlings 6 weeks old grown in Courtland greensand.
B.—Corn seedlings 6 weeks old grown in Courtland greensand to which had been added lime carbonate at the rate of 4 tons per acre.

A

B